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Indoor Residual Spraying (IRS 2) Task Order Six

AIRS KENYA ENTOMOLOGICAL MONITORING REPORT

DECEMBER 2015 – SEPTEMBER 2017

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AIRS KENYA ENTOMOLOGICAL MONITORING REPORT

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EXECUTIVE SUMMARY

Control of malaria vectors depends mainly on use of long lasting insecticidal nets (LLINs) and application of indoor residual spraying (IRS). These interventions have been proven to be effective in controlling malaria transmission. However, their sustained use may elicit changes such as physiological or behavioral resistance in malaria vectors. Such changes in vector bionomics may limit the effectiveness of interventions and hinder the achievement of their operational goals. Routine monitoring of mosquito densities, species composition, biting time and location, sporozoite infection status and insecticide susceptibility status provide critical information to assess progress in meeting the operational goals of a vector control program.

PMI-AIRS Kenya, in collaboration with the Kenya National Malaria Control Program (NMCP), conducted IRS with pirimiphos-methyl CS in Migori County, western Kenya between February and March 2017. To monitor the impact of IRS, AIRS implemented monthly entomological monitoring by pyrethrum spray catches (PSC), CDC light traps, window exit traps and outdoor drums to monitor indoor resting, biting, exiting and outdoor resting rates, respectively. Additional surveillance was conducted to assess vector biting behavior and insecticide susceptibility status before and after IRS. Following application of IRS, evaluations were performed to assess quality of spray and longevity of the insecticide.

This report describes results of entomological surveillance conducted from December 2015 up to September 2017. Before IRS, malaria vector composition in the study area was 80% *An. funestus*, 17% *An. gambiae* s.l., 1% *An. coustani* and <1% other *Anopheles* species. *An. funestus* was the most dominant vector in both IRS and non-IRS sites before IRS with 84% and 86%, respectively. However, after IRS, *An. gambiae* s.l. became the dominant species (79%) in the spray area, while *An. funestus* was still highest (76%) in the control sites. Densities of *An. funestus* post-spray were significantly reduced in the IRS sites as compared to non-IRS by 95% (P<.0001) and 97% (P<.0001) in light trap and PSC, respectively. However, no significant reduction was observed between IRS and non-IRS sites after spray in the density of *An. gambiae* s.l. by light trap while only marginal reduction, 48% (P=0.05) was observed by PSC. Molecular species identification showed the majority of *An. gambiae* s.l. were *An. arabiensis*. The risk of exposure to mosquito bites was greatly reduced with very few vectors collected after IRS by HLC. In addition, no sporozoite infection was detected in mosquitoes from the intervention sites post-IRS. Pirimiphos-methyl (Actellic 300CS®) used in spraying showed prolonged potency, killing over 80% of the susceptible *An. gambiae* s.s up to 9 months post spray. Additionally, the local vector population showed full susceptibility to pirimiphos-methyl, chlofenapyr and clothianidin.

The results show rapid reduction of *An. funestus* numbers in the spray sites with insignificant impact on *An. arabiensis* populations following one round of IRS with Actellic 300CS®. Reduction of *An. funestus* population reduces the risk of exposure to mosquito bites and malaria transmission. However, the low impact on *An. arabiensis* is possibly due to the behavior of the vector leading to avoidance of indoor interventions.

1. INTRODUCTION

Over the last two decades, malaria control in much of sub-Saharan Africa has focused on scale up of LLINs and application of IRS to populations at risk. In Kenya, distribution of mosquito nets began in late 2001 with the sale of subsidized conventional nets bundled with insecticide treatment (deltamethrin) through rural retail shops [1]. The following years saw the initiation of distribution of subsidized nets through antenatal clinics (ANCs) to pregnant mothers and children under 5 years [1]. In 2006, the government initiated a mass net distribution campaign achieving a coverage of 58% of houses with at least one ITN and 28% with more than one net in the malaria endemic regions [1]. Recently, a remarkable increase in the ownership and use of LLINs has been achieved in the country with the highest net coverage in western Kenya. The revised Kenya Malaria Strategy (KMS) set a target of 80% of the at-risk population using appropriate malaria prevention interventions, including ITNs and IRS, by 2018. The Government of Kenya objective is to achieve universal ITN coverage (i.e., one net for every two people) for all groups in malaria-endemic and epidemic-prone counties through: (1) regular rolling mass ITN distribution campaigns, carried out every three years in targeted geographic areas; (2) routine distribution through antenatal care (ANC) and Expanded Program on Immunization (EPI) clinics in 36 counties; (3) social marketing of nets particularly in designated rural counties; and (4) commercial sales of ITNs in the private sector. The revised KMS has prioritized IRS for malaria-endemic counties with additional support for capacity building and focal IRS in epidemic-prone counties [2].

Unlike LLIN distribution, application of IRS in Kenya has been conducted only in a few counties. Spraying was initially focused in 12 epidemic prone counties and 3 endemic counties as an epidemic response measure following appropriate signals from an early warning system [3]. In 2007 a more systematic approach was adopted as an epidemic response. The focus changed between 2010 and 2012 to cover entire counties with IRS, starting in Homa Bay, Migori and parts of Kisumu counties, in the Lake Victoria malaria endemic region of western Kenya. However, spraying was interrupted between 2013 and 2017 due to the detection of widespread pyrethroid resistance in the vector population in the region and the lack of a registered non-pyrethroid insecticide in the country. In 2017, IRS was re-introduced in Migori County using pirimiphos-methyl.

The global community has recommended the development of insecticide resistance management plans in each country to preserve the available insecticides [4]. Consequently, in Kenya, the NMCP has developed an insecticide resistance management strategy involving rotation of different classes of insecticides every 2 years in IRS implementation [5].

Vector surveillance is important in the evaluation of any vector control program to measure impact or process indicators against operational targets and objectives [6]. Entomological surveillance is therefore necessary to understand the progression towards malaria control in relation to entomological inoculation rates (EIR), mosquito densities, vector species composition and distribution, human feeding rates, vector behaviour and population structure. Vector surveillance also monitors susceptibility of malaria vectors to insecticides. Consequently, the US President's Malaria Initiative – Africa Indoor Residual Spray (PMI-AIRS) program has conducted vector surveillance in parts of Migori and Homa Bay counties in western Kenya to evaluate the IRS campaign with pirimiphos-methyl recently conducted in Migori County.

The current report is the third covering surveillance in nine sub counties, seven in Migori and two in Homa Bay County, and expands the period of data collection to 22 months, 15 months before and 7 months after spray (December 2015 to September 2017) to test the impact of IRS on the local vector population. The AIRS-Kenya program monitored indoor resting densities, indoor biting rates, exiting rates and outdoor resting vector populations. Other investigations include resistance monitoring to guide choice of insecticide for IRS and the time and location of mosquito biting.

Results from this study are important in decision making by the NMCP and other development partners in the fight against malaria.

1.1 Main Objective

To determine the efficacy of IRS with pirimiphos-methyl in control of malaria vectors in the endemic counties of western Kenya.

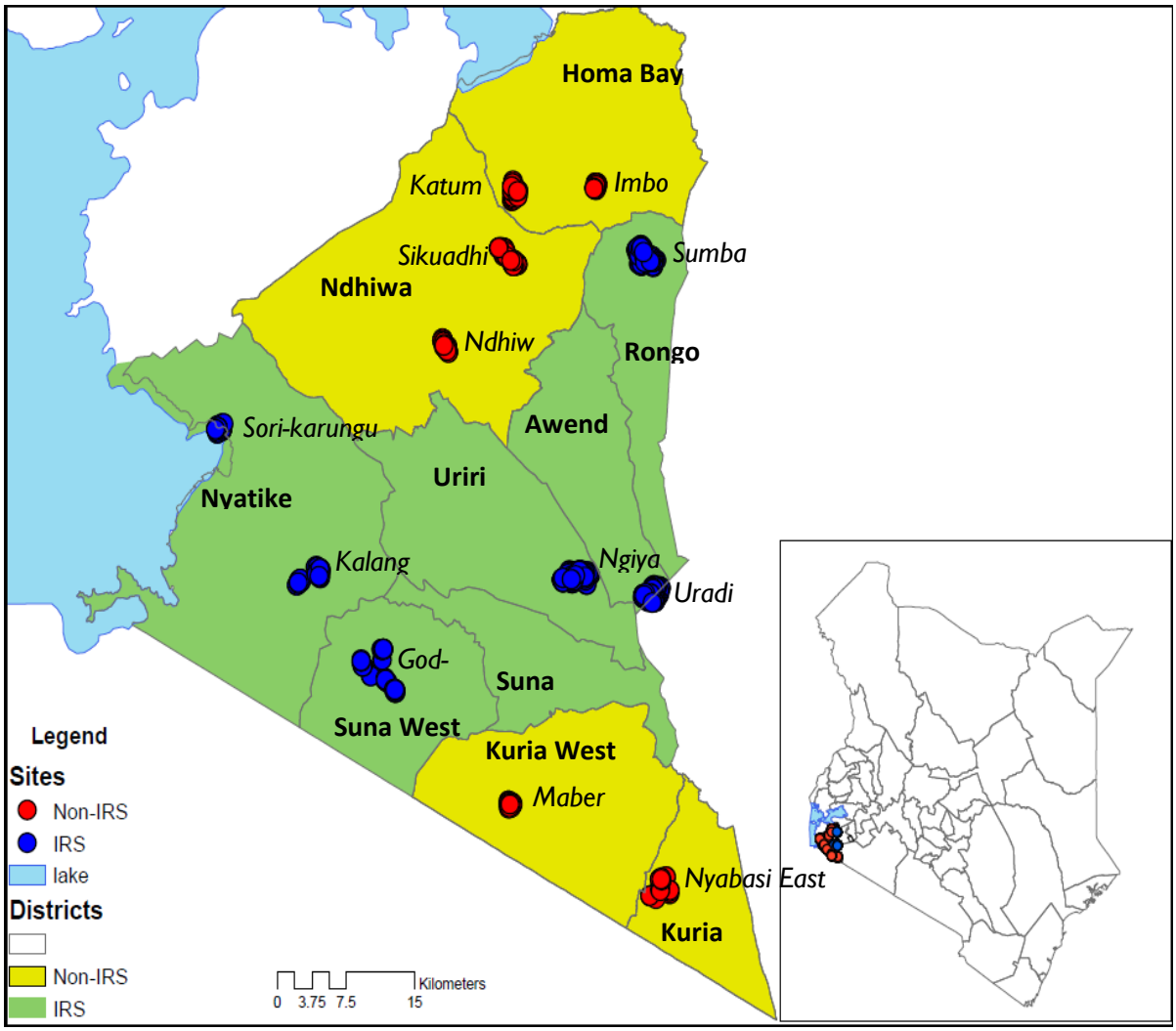
1.2 Specific Objectives

I. To monitor malaria vector density in Migori County before and after implementation of IRS
II. To determine and monitor the levels and mechanisms of insecticide resistance of local malaria vector populations
III. To determine the decay rate of insecticide on walls following the IRS campaign
IV. To determine the impact of IRS on vector behavior
V. To compare community entomological monitoring with AIRS standard monitoring in Siaya County

2. METHODOLOGY

2.1 STUDY SITE

FIGURE 1: MAPS SHOWING IRS AND NON-IRS SUB COUNTIES AND THE DISTRIBUTION OF SURVEILLANCE SITES IN MIGORI AND HOMA BAY COUNTIES[1].



Entomological monitoring was conducted in 12 sites, six in the IRS area and six in unsprayed regions (Figure 1). In Migori County, monitoring was conducted in Rongo, Awendo, Uriri, Suna West and Nyatike Sub Counties, all of which received IRS with pirimiphos-methyl in February-March 2017. Additional sites in Migori were Kuria West and Kuria East Sub Counties which did not receive IRS. There were also two unsprayed comparison sub counties in Homa Bay County, Homa Bay and Ndhiwa Sub Counties with two sites each (Table 1). All the sites were in a malaria hyperendemic region of western Kenya with Nyatike and Homa Bay bordering Lake Victoria while Rongo and Awendo border the highlands.

[1] Data was collected from dropped sites between December 2015 and May 2016.

TABLE 1: ENTOMOLOGICAL SURVEILLANCE SITES AND KEY INDICATORS

Sub county	Sentinel Site Location	Intervention Status	Collection period	Data Collected
Rongo	Sumba	IRS	Dec 2015 – Sep 2017	Species composition, resistance testing and monitoring, vector density surveillance, mosquito behavior, insecticide residual life
Awendo	Uradi	IRS	Dec 2015 – Sep 2017	
Uriri	Ngiya	IRS	Dec 2015 – Sep 2017	
Suna West	God-kwer	IRS	Jul 2016-Sep 2017	
Nyatike	Sori-Karungu	IRS	Jul 2016-Sep 2017	
Nyatike	Kalangi	IRS	Jul 2016-Sep 2017	
Kuria West	Mabera	Control	Jul 2016-Sep 2017	Species composition, resistance testing and monitoring, vector density surveillance, mosquito behavior
Kuria East	Nyabasi East	Control	Jul 2016-Sep 2017	
Homa Bay	Imbo	Control	Jul 2016-Sep 2017	
Homa Bay	Katuma	Control	Dec 2015 – Sep 2017	
Ndhiwa	Sikwadhi	Control	Jul 2016-Sep 2017	
Ndhiwa	Ndhiwa	Control	Dec 2015 – Sep 2017	

2.2 VECTOR DENSITY SURVEILLANCE

Mosquito collections for population monitoring were conducted every month in all sites by PSC, indoor light trap, window exit trap and outdoor drum collections. During mosquito collection, household information including roof type, wall type, presence of eaves, presence of nets, number under net, number not under net, and presence of cattle were collected on a tablet.

2.3 INDOOR RESTING RATES

Indoor-resting mosquitoes were collected by morning pyrethrum spray catches (PSC) in five houses per site per month. New houses were randomly selected each month. A verbal consent was sought from the household head or a legal representative before collections were performed. On consenting, the household head was requested to either remove or cover any food items and prepare the house for PSC. Mosquito collection was done by laying white sheets on the floor and over the furniture within the house. Two collectors, one inside the house and another outside, sprayed around the eaves with 0.025% pyrethrum emulsifiable concentrate mixed with 0.1% piperonyl butoxide in kerosene. The collector inside the house then sprayed in the roof space. The house was closed for 10-15 minutes after which knocked-down mosquitoes were collected from the sheets and transferred to the laboratory in a scintillation vial containing 70% ethanol.

2.4 INDOOR HOST SEEKING RATES

Mosquitoes seeking hosts indoors at night were collected by CDC light traps in 10 houses per site per month. New houses were randomly selected each month. A single 12 V CDC light trap was hung in each house in the sleeping area, approximately 1.5 m from the ground, adjacent to an occupied bed net. The traps were run from 06:00 pm and mosquitoes were collected at 07:00 am the next morning. The trapped mosquitoes were transferred into paper cups and transported to the laboratory for further analysis.

2.5 EXITING RATES

Mosquitoes exiting houses before morning were collected using window exit traps installed in 5 houses in each site per month, excluding sites in Kuria East and Kuria West (because there were no windows in most houses). New houses were randomly selected each month and the same houses were used for window exit trap and PSC. In each house sampled, a single exit trap was installed on a window in the sleeping area. The window trap was fitted in the window space with funnel shaped entry point facing the house and the trap was supported by an adjustable metallic stand from below. The trap was surveyed early the following morning and trapped mosquitoes were transferred into paper cup using mouth aspirators. The samples were taken to the laboratory for further analysis.

2.6 Outdoor Resting Rates

To monitor outdoor resting mosquito populations, 10 plastic drums were distributed at each sentinel site. Each drum contained heavy cloth material at the bottom that was kept wet to maintain the inside cool and humid. The drums were cut on the sides to allow ease of access by mosquitoes. They were placed outdoor in shaded areas with minimal interference. The drums were kept permanent at their locations and were examined once a month for mosquitoes resting inside. These mosquitoes were collected from the drums by prokopack aspirators, transferred into an adult mosquito cage, and sorted into paper cups for transportation to the laboratory.

2.7 Quality of Spray and Decay Rate of Insecticide on the Wall

To determine the quality of spray and rate of decay of the insecticide on the sprayed walls, cone wall bioassays were conducted within two weeks of IRS and continued monthly using a susceptible colony of *An. gambiae* s.s. Kisumu strain. Bioassays were conducted using the WHO cone bioassay technique in 10 randomly selected houses in four clusters within the spray area following the commencement of the IRS campaign. Subsequent exposures were performed in the same houses monthly.

The exercise was conducted in Rongo, Uriri, Nyatike and Suna West Sub Counties in Migori County. In each sub county, a village with already sprayed houses was identified for wall bioassays. In every village, seven houses of mud and three with cement walls were selected for cone bioassay. Each site used the same distribution by wall type to reduce bias. In each sprayed house, 10 laboratory-reared, 2-5 day old, non-blood fed female *Anopheles gambiae* Kisumu strain mosquitoes were exposed for 30 minutes on three different walls at varying heights: 0.5 m, 1.0 m and 1.5 m from the floor. A fourth cone was used to expose field collected adult *An. funestus* alongside the susceptible colony at a height of 1 m from the floor. Due to the difficulty of raising *An. funestus* or collecting sufficient numbers for experiments, only 10 mosquitoes were exposed per house whenever the samples were available. Temperature and relative humidity were recorded at every house where mosquitoes were exposed. A control cone of lab-reared *An. gambiae* was set on a plywood board outside of each sprayed house in a shaded area close to the house. Wild *An. funestus* were not used in the control due to the low numbers.

2.8 Human Biting Rates

Human landing catches (HLC) were used to assess biting time and location (indoor vs outdoor) of the local vector population before and after spraying. The collections were conducted in Rongo, Awendo, Uriri and Sori Karungu in the spray area and Ndhiwa and Homa Bay in the non-spray sites. In each cluster, five houses were identified and consent was obtained for mosquito collection. Collectors were recruited from among residents of each cluster. All collectors gave consent and were tested for malaria at least seven days before collection begun, and any testing positive received full malaria treatment according to the national guidelines. Those who tested negative started malaria prophylaxis with mefloquin one week before the start of collections, while those who initially tested positive started prophylaxis with mefloquin two weeks after initial treatment. All collectors took repeat doses every 7 days for six weeks. Over the same period, the collectors were monitored for malaria infection and anyone who presented with malaria related symptoms was referred for testing and treatment. No case of malaria infection was reported among the collectors within the collection and follow up weeks period.

During HLC, one volunteer sat outside within 5 m from the house and another sat inside the house in the living room. Collectors kept their trousers folded to knee length and aspirated any mosquitoes landing on them. Each house had a team of six collectors, each working in one of three shifts running from 5 p.m. to 11 a.m. the next morning. Each shift was for 6 hours. Collections were performed for 45 minutes and the collectors rested for 15 minutes in each collection hour. At the end of each collection hour, the collectors recorded location of members of the household. Collected mosquitoes were separated by time and location and sustained on 10% sugar solution before being transported to the laboratory for analysis.

2.9 Insecticide Resistance Monitoring (WHO Cylinder Tests)

Mosquito collections for insecticide resistance monitoring were performed between June and September 2017. Larval stages of *An. gambiae* s.l. were collected from Homa Bay and Ndhiwa sub-counties of Homa Bay county; Rongo and Nyatike sub-counties in Migori county; and Rarieda sub-county in Siaya County. Collections were performed using larval dippers and sieves. *Anopheles* larvae were separated from the other aquatic organisms and sorted into different larval instars. The larval samples were maintained in a room with a portable space heater while in the field and were fed on fish meal. Pupae developing from the larvae were collected daily and placed in pupal cups. The pupal cups were then introduced into paper cups labelled with the collection site and provided with a wet cotton wool soaked in 10% sugar solution. The emerging adults were trapped in the paper cups and sustained on the provided sugar pad. The emergent adults were marked with the date of emergence and raised to three-day old adults for insecticide resistance tests.

Adult mosquitoes were also collected for insecticide resistance tests, especially where larval sources were few. Collections were performed by hand aspiration inside houses in Homa Bay and Ndhiwa sub-county of Homa Bay county; and Bumula and Rarieda sub-counties of Bungoma and Siaya counties respectively. Collected mosquitoes were placed in paper cups and labelled with the collection site and date. All collected mosquitoes were transported to a holding room in the field and were monitored for 24 hours before insecticide resistance tests were performed. It was not possible to get adult *An. funestus* s.l. in Rongo, Awendo and Uriri sub-counties in Migori county due to low numbers of adult mosquitoes after IRS, while *An. gambiae* s.l. larvae were collected only in Rongo sub-county.

Insecticide resistance status was assessed using the WHO test-tube bioassay using diagnostic concentrations of deltamethrin (0.05%), permethrin (0.75%), pirimiphos-methyl (0.25%) and alpha-cypermethrin (0.05%). All papers were prepared by the WHO collaborating centre, University Sains Malaysia. Insecticide susceptibility testing was also conducted for clothianidin (neonicotinoid) and chlorfenapyr (pyrrole), which are both new insecticides that recently received WHO recommendation for IRS and LLINs respectively. WHO is in the process of determining diagnostic concentrations for these new insecticides. Therefore, the clothianidin dosage was determined based on internal testing conducted by Sumitomo which showed 2% w/v clothianidin to be a suitable diagnostic concentration for each treated filter paper. For chlorfenapyr, CDC bottle bioassay is the preferred method, and CDC has proposed a diagnostic dose of 100 µg/bottle. Due to variable results using this concentration in other countries, several doses were tested around this dose at 12.5, 25, 50, 100 and 200µg/bottle.

The WHO bioassay was done using 2- to 5-day-old *An. gambiae* s.l. emerging from collected larvae or by direct exposure of collected adult *An. funestus.* At least 100 mosquitoes of each species were exposed to each insecticide at a time. Knock-down was monitored every 10 minutes for 60 minutes. The samples were then transferred to a holding tube with cotton wool soaked in sugar solution and held for 24 hours. Mortality was scored 24 hours after exposure.

Clothianidin tests were conducted using filter papers prepared by AIRS staff. Whatman® No.1 filter papers were treated with the diagnostic dose of clothianidin according to AIRS standard operating procedure 001. The treated papers were stored in aluminum foil at 4°C. The treated filter papers were inserted into the holding tubes and the tests were conducted according to the WHO susceptibility test protocol. Up to 25 mosquitoes were exposed at a time for 60 minutes after which the mosquitoes were transferred to a holding tube and mortality was monitored up to 7 days post exposure. Treated papers were tested within 24h of preparation.

Chlofenapyr exposures were done using the CDC bottle assay. Mosquitoes were exposed for 1h, with results being recorded immediately after the end of the test and subsequently every 24h for three days to record any delayed mortality. Conditions were strictly kept at 28±1°C due to published findings of temperature sensitivity during daytime bioassays with chlorfenapyr [7].

2.10 Molecular Analysis

All vectors collected were identified morphologically to species [9, 10]. The physiological status was determined by observation of the abdomen to classify the female mosquitoes as either fed, gravid, half gravid or unfed. Female mosquitoes were divided into three parts for various procedures; head and thorax was used for determination of sporozoite rate by enzyme linked immunosorbent assay (ELISA) techniques (Wirtz et al 1987), the abdomen of blood-fed and half-gravid females were kept for blood-meal host determination and the remainder of the specimen was used in polymerase chain reaction (PCR) analysis to identify members of the *An. gambiae* s.l. and the *Anopheles funestus* s.l. groups [11] and for future genetic/molecular analysis. Blood meal PCR is ongoing and will be reported separately. All mosquitoes morphologically identified as *An. gambiae* s.l. were analyzed by PCR for species identification while a random selection of 20% of *An. funestus* s.l., were initially analyzed for species identification in each month.

2.11 Data Management

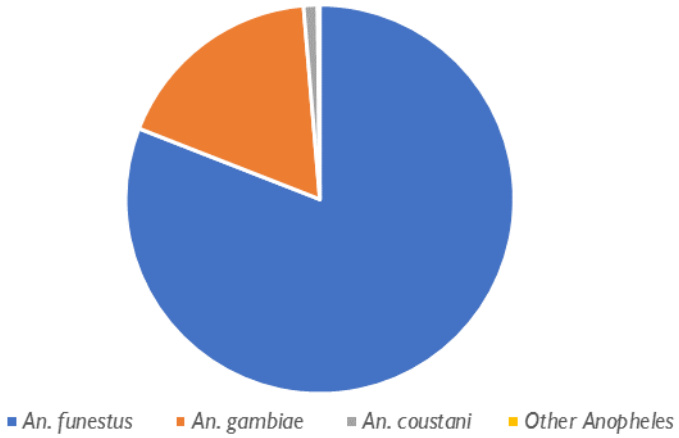
Data collection was done using open data kit software (ODK) run on tablets. The data collection interface was designed with buttons, drop down menus, and data quality checks to limit entry errors in the field. Each house sampled was allocated a unique code and a study number. Collection devices containing mosquitoes from each house were marked with these numbers and the numbers were used to track the samples through all the laboratory procedures. Individual mosquitoes were labelled with pre-printed barcodes and linked to the field data by house code and a unique study number at data management level. Additional tests on individual mosquitoes, including sporozoite ELISAs and species identification by PCR, were linked by the unique barcode label. Data entry screens used drop down menus and automatic data checks to reduce errors. For data sharing, all data was merged into a single file and checked to ensure a proper merge. Data was fitted using Generalized Linear Mixed Effects statistical Models (GLMMs) with negative binomial distribution in SAS (Version 9.2) to test changes in *Anopheles* densities before and after IRS.

3. RESULTS

3.1 SPECIES COMPOSITION

A total of 9,816 *Anopheles* mosquitoes; 8,525 females and 1,291 males were collected by all methods combined. Only female mosquitoes were used in the analysis. The overall species composition by morphological identification was 80% *An. funestus* s.l., 17% *An. gambiae* s.l., 1% *An. coustani* and <1% other *Anopheles* (*An. maculipalpis, An. pharoensis* and *An. rufipes*) (Figure 2).

FIGURE 2: OVERALL % *ANOPHELES* SPECIES COMPOSITION BY MORPHOLOGICAL IDENTIFICATION, DECEMBER 2015 TO SEPTEMBER 2017.



An. funestus s.l. was the predominant malaria vector before the spray campaign, representing 84% of *Anopheles* caught in IRS-designated sites and 86% in non-IRS sites. After IRS, 79% of *Anopheles* samples were *An. gambiae* s.l. in the sprayed area, while *An. funestus* s.l. (76%) remained the predominant species in the unsprayed control sites (Figure 3).

Molecular species identification by PCR showed those mosquitoes identified as *An. gambiae* s.l. to be mostly (832 of 840 or 99%) *An. arabiensis*, while only 8 of 840 (1%) were *An. gambiae* s.s. PCR analysis of 3,692 mosquitoes morphologically identified as *An. funestus* s.l. confirmed that all were An. *funestus* s.s. (Table 2)

FIGURE 3: % VECTOR SPECIES COMPOSITION PRE-IRS (DECEMBER 2015 TO FEBRUARY 2017) AND POST-IRS (MARCH TO SEPTEMBER 2017) IN IRS AND UNSPRAYED SITES.

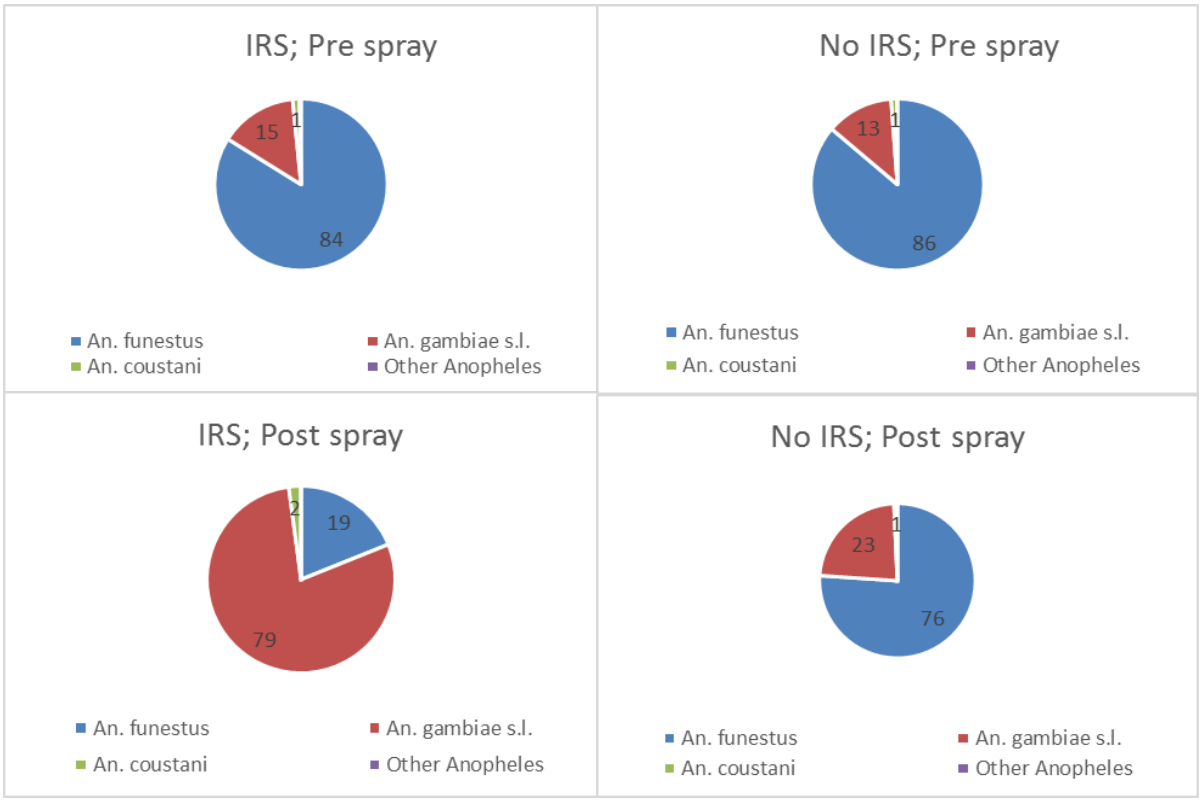


TABLE 2: *ANOPHELES* SPECIES COMPOSITION BY MORPHOLOGICAL IDENTIFICATION AND SPOROZOITE RATES ACCORDING TO SPRAY STATUS, SUB-COUNTY, AND TIME PERIOD

		An. funestus			An. gambiae s.l.			An. coustani			Other Anopheles			Sporozoite rate, all *Anopheles*	
IRS Status	**Sub county**	**Pre spraying**	**Post spray**	**Total**	**Pre spray**	**Post spray**	**Totals**	**Pre spray**	**Post spray**	**Totals**	**Pre spray**	**Post spray**	**Totals**	**Pre spray**	**Post spray**
IRS	Awendo	852 (98)	19 (2)	871	199 (78)	56 (22)	255	39 (93)	3 (7-)	42	0	0	0	0.027	0
	Nyatike	55 (86)	9 (14)	64	155 (68)	73 (32)	228	0	1 (100)	1	0	0	0	0.005	0
	Rongo	1495 (99)	9 (1)	1504	183 (77)	54 (23)	237	17 (100)	0	17	0	0	0	0.03	0
	Suna west	15 (88)	2 (12)	17	7 (39)	11 (61)	18	0	0	0	0	0	0	0.048	0
	Uriri	1722 (99)	15 (1)	1737	173 (84)	32 (16)	205	7 (78)	2 (22-)	9	14 (100)	0	14	0.028	0
	Sub total	4139 (99)	54 (1)	4193	717 (76)	226 (24)	943	63 (918)	6 (9)	69	14 (100)	0	14	0.028	0
No IRS	Ndhiwa	841 (56)	659 (44)	1500	126 (43)	170 (57)	296	10 (77)	3 (23)	13	0	0	0	0.044	0.030
	Homa Bay	697 (70)	292 (30)	989	108 (44)	136 (56)	244	11 (100)	0	11	1 (100)	0	1	0.040	0.037
	Kuria	83 (38)	134 (62)	217	3 (11)	25 (89)	28	0	7 (100)	7	0	0	0	0	0.006
	Sub total	1621 (60)	1085 (40)	2706	237 (42)	331 (58)	568	21 (68)	10 (3226)	31	1 (100)	0	1	0.042	0.03

*Pre-spray period covered December 2015 to February 2017 while post-spray period covered March 2017 to September 2017.

3.2 VECTOR SEASONALITY

3.3 INDOOR HOST SEEKING *ANOPHELES* SPECIES DENSITIES PRE- AND POST-IRS

Indoor host-seeking densities of *An. funestus* over time showed peaks corresponding to periods of the long and short rains (Figure 4). While the trends were similar pre-IRS in all sites, there were significantly more *An. funestus*, (mean =1.83 (1.541-2.118), per CDC-LT observed in sites designated for IRS during the pre-IRS period than control sites at 1.16 (0.958-1.361) per CDC-LT, P=0.0001(Figure 5). The densities of *An. funestus* were reduced by IRS so much that there were no seasonal peaks in IRS regions post-spray (Figure 4). *An. funestus* densities were reduced by 95% in the IRS site as compared to non-IRS sites after IRS, with a mean of 0.067 per night (0.032-0.103) in IRS sites and 1.32 (1.014-1.625) in unsprayed sites (P<0.0001) (Figure 5).

FIGURE 4: MONTHLY MEAN NUMBER OF INDOOR HOST SEEKING *AN. FUNESTUS* PRE- AND POST-SPRAY IN BOTH IRS AND NON-IRS SITES (COLLECTED USING INDOOR CDC LIGHT TRAP).

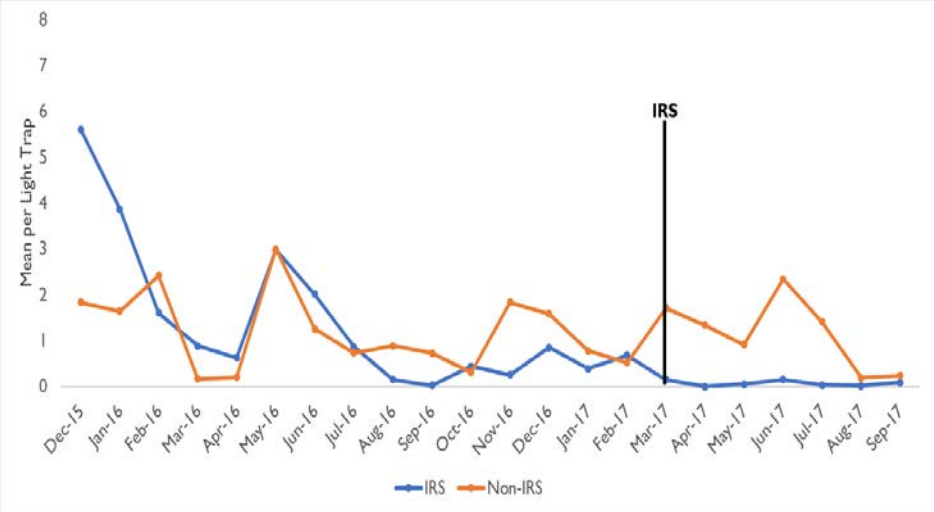
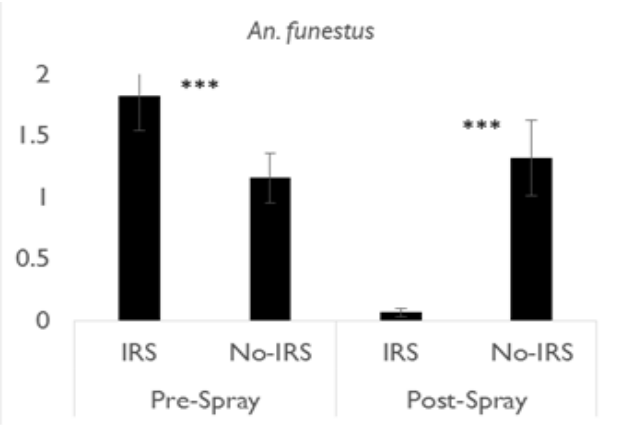


FIGURE 5: DENSITY OF INDOOR HOST SEEKING *AN. FUNESTUS* PRE- AND POST-SPRAY IN IRS AND NON-IRS SITES



The densities of *An. gambiae* s.l. were generally much lower compared to *An. funestus* before IRS (Figure 6). *An. gambiae* s.l. densities were significantly higher in IRS sites as compared to non-IRS sites before spraying at 0.339 (0.273-0.406) per CDC-LT compared with 0.136 (0.093-0.180),

P<0.0001 (Figure 7). Subsequently, an insignificant 19% reduction in the vector density was observed in sprayed areas compared to unsprayed sites after IRS (Table 3). However, further analysis is needed to take into account the pre-spray IRS differences. For total *Anopheles*, densities were significantly different before IRS with a mean of 2.22 (1.907-2.544) per CDC-LT in IRS designated sites and 1.32 (1.111-1.535) in control sites (P<0.0001). However, following IRS, indoor host seeking *Anopheles* densities were 81% lower in IRS sites than control sites (Table 3).

FIGURE 6: MONTHLY MEAN NUMBER OF INDOOR HOST SEEKING *AN. GAMBIAE* S.L. PRE- AND POST-SPRAY IN BOTH IRS AND NON-IRS SITES (COLLECTED USING INDOOR CDC LIGHT TRAP)

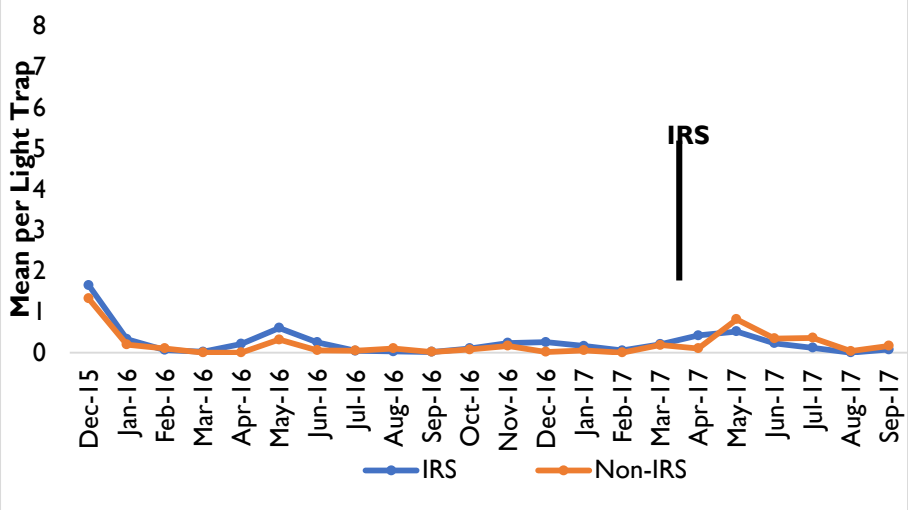


FIGURE 7: DENSITY OF INDOOR HOST SEEKING *AN. GAMBIAE* S.L. PRE- AND POST-SPRAY IN IRS AND NON-IRS SITES

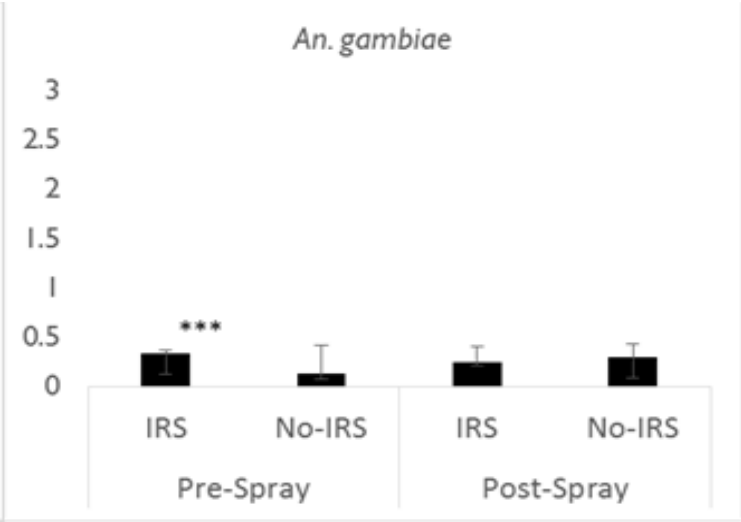


TABLE 3: COMPARISON OF DIFFRENCES IN TOTAL ANOPHELES, *AN. FUNESTUS* AND *AN. GAMBAIE* S.L. MEAN INDOOR HOST SEEKING DESITIES BEFORE AND AFTER IRS IN THE SPRAYED AND NON-SPRAYED SITES

	Period of comparison	Intervention status	Trapping nights	Mean	% difference in Anopheles density	P
Total Anopheles	Pre-IRS	IRS	1176	2.2253	+69	<0.0001
		No IRS	733	1.3233		
	Post-IRS	IRS	356	0.3315	-81	<0.0001
		No IRS	347	1.6513		
An. funestus	Pre-IRS	IRS	1176	1.8291	+59	0.0001
		No IRS	733	1.1596		
	Post-IRS	IRS	356	0.0674	-95	<0.0001
		No IRS	347	1.3199		
An. gambiae s.l.	Pre-IRS	IRS	1176	0.3393	+50	<0.0001
		No IRS	733	0.1364		
	Post-IRS	IRS	356	0.2472	-19	0.4923
		No IRS	347	0.3055		

3.4 INDOOR RESTING *ANOPHELES* SPECIES DENSITIES PRE- AND POST-IRS

The trends for indoor pyrethrum spray catch were similar to CDC-light trap with a remarkable decrease in the resting densities of *An. funestus* after IRS (Figure 8 and 9). Monthly trends of indoor resting *An. funestus* showed clear peaks of periods of high vector densities corresponding to the end of the long and short rains in all sites during baseline data collection.

The period following IRS showed a marked reduction in the indoor resting *An. funestus* densities in the IRS sites as compared to non-IRS sites (Figure 9). Mean indoor resting density of *An. funestus* was not significantly different between the IRS and non-IRS sites during baseline (P=0.084). After IRS there were 97% fewer *An. funestus* captured in IRS sites compared to non-IRS sites (P<0.0001) (Figure 9 and Table 4).

Indoor resting *An. gambiae* s.l. densities were low throughout the study period and there was no significant difference in the mean number of the species between IRS and non-IRS sites pre-spray (Figure 10). However, a marginally significant reduction in mean indoor resting density was observed in IRS areas compared to unsprayed areas (P=0.0468) post IRS (Figure 11 and Table 4). Overall the mean density of indoor resting *Anopheles* mosquitoes was 95% lower in the IRS sites compared to non-IRS sites, P<0.0001 (Table 4).

FIGURE 8: MONTHLY MEAN NUMBER OF INDOOR RESTING *AN. FUNESTUS* PRE- AND POST-SPRAY IN BOTH IRS AND NON-IRS SITES (COLLECTED BY PSC).

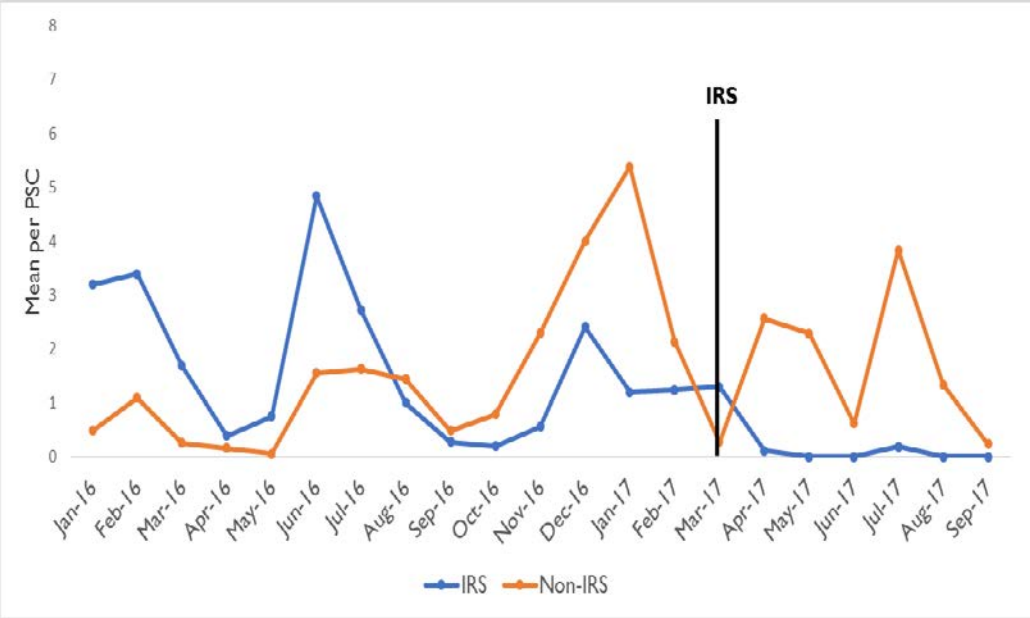


FIGURE 9: DENSITY OF INDOOR RESTING *AN. FUNESTUS* PRE- AND POST-SPRAY IN BOTH IRS AND NON-IRS SITES

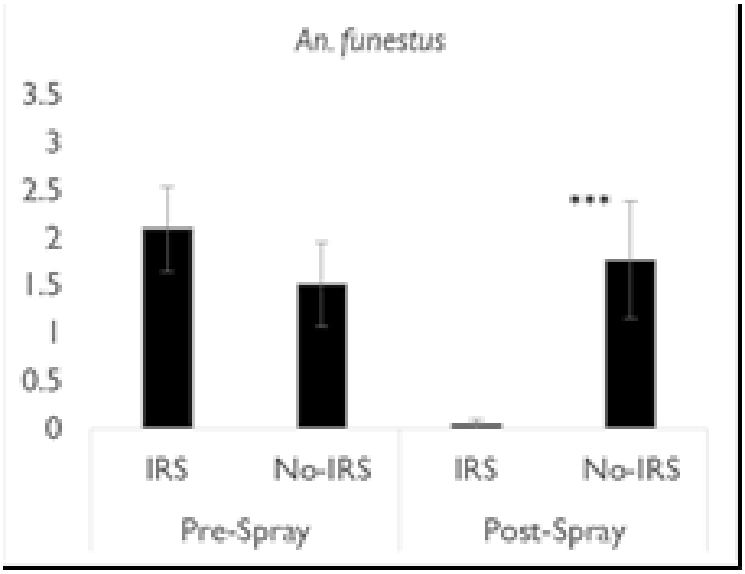


FIGURE 10: MONTHLY MEAN NUMBER OF INDOOR RESTING *AN. GAMBIAE* S.L. PRE- AND POST-SPRAY IN BOTH IRS AND NON-IRS SITES (COLLECTED BY PSC).

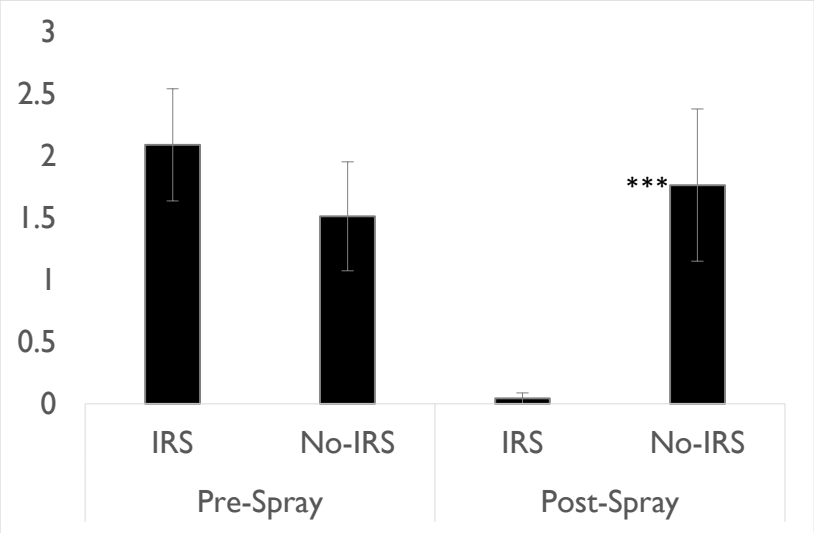


FIGURE 11: DENSITY OF INDOOR RESTING *AN. GAMBIAE* S.L PRE- AND POST-SPRAY IN BOTH IRS AND NON-IRS SITES

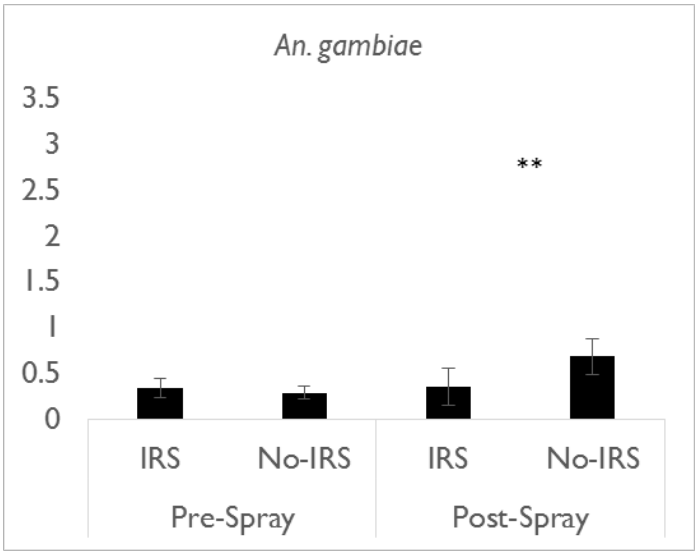


TABLE 4: COMPARISON OF TOTAL *ANOPHELES*, *AN. FUNESTUS* AND *AN. GAMBIAE S.L.* MEAN INDOOR RESTING DENSITIES BEFORE AND AFTER IRS IN SPRAYED AND NON-SPRAYED SITES.

	Period of comparison	Intervention status	Trapping nights	Mean	% difference in Anopheles density	P
Total Anopheles	Pre-IRS	IRS	835	2.4359	+35	0.0639
		No IRS	446	1.8027		
	Post-IRS	IRS	225	0.4	-95	<0.0001
		No IRS	239	2.4477		
An. funestus	Pre-IRS	IRS	835	2.091	+37	0.0837
		No IRS	446	1.5135		
	Post-IRS	IRS	225	0.0444	-97	<0.0001
		No IRS	239	1.7657		
An. gambiae s.l.	Pre-IRS	IRS	835	0.3401	+18	0.4047
		No IRS	446	0.287		
	Post-IRS	IRS	225	0.3556	-48	0.0468
		No IRS	239	0.682		

3.5 *ANOPHELES* EXITING RATES PRE-AND POST IRS

Monthly mean exiting rates of *Anopheles* mosquitoes in both IRS and non-IRS sites were largely similar in the pre-IRS period. However, in the post-IRS period, exiting rates were markedly reduced in the spray sites as compared to unsprayed sites (Figure 12 and Table 5). Exiting rates of *An. gambiae* s.l. were consistently low in both IRS and non-IRS sites before IRS. However, mean exiting densities were increased in the post spray period. Similar trends were observed with *An. funestus,* high exiting rates post spray.

In a comparison of *Anopheles* exiting rates between IRS and non-IRS sites before spray, no significant difference was observed. However, in the post spray period, significantly fewer *Anopheles* mosquitoes, 90% fewer, were trapped exiting in in the sprayed sites as compared to unsprayed sites, p<.0001. Similarly, *An. gambiae* s.l. and *An. funestus* showed significant differences in exiting rates between sprayed and unsprayed sites after spraying.

FIGURE 12: MONTHLY MEAN NUMBER OF EXITING *ANOPHELES* PRE- AND POST-SPRAY IN BOTH IRS AND NON-IRS SITES (COLLECTED BY WINDOW EXIT TRAP-WET)

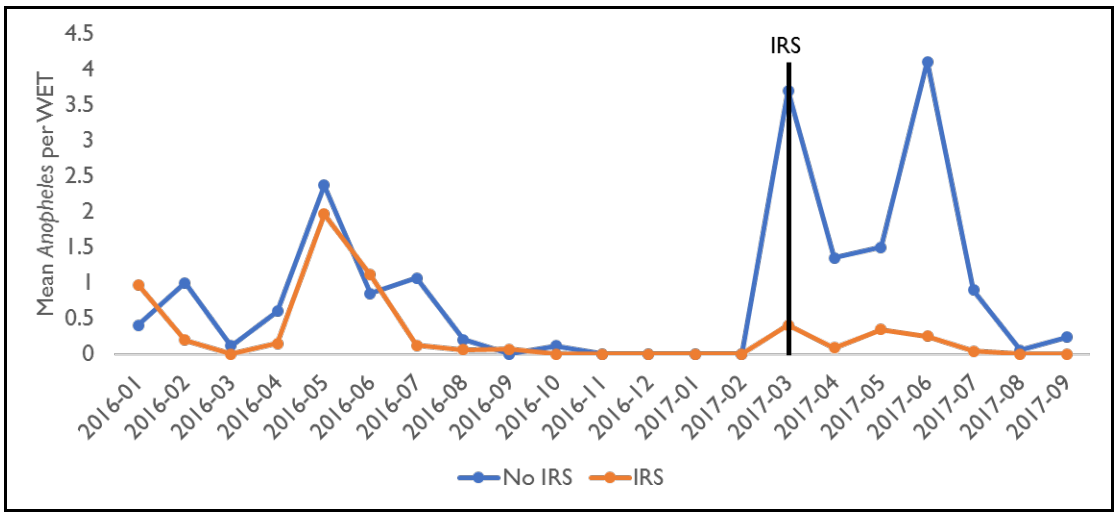


TABLE 5: COMPARISON OF MEAN EXITING DENSITIES OF TOTAL *ANOPHELES*, *AN. FUNESTUS* AND *AN. GAMBIAE* S.L. BEFORE AND AFTER IRS IN SPRAY AND NON-SPRAY SITES.

	Period of comparison	Intervention status	Trapping nights	Mean	% difference in Anopheles density	P
Total Anopheles	Pre-IRS	IRS	495	0.5455	16	0.5833
		No IRS	255	0.4784		
	Post-IRS	IRS	166	0.1928	90	<0.0001
		No IRS	119	1.8992		
An. funestus	Pre-IRS	IRS	495	0.4889	14	0.6579
		No IRS	255	0.4313		
	Post-IRS	IRS	166	0.0843	94.43	<0.0001
		No IRS	119	1.5126		
An. gambiae s.l.	Pre-IRS	IRS	495	0.0545	25	0.5725
		No IRS	255	0.0431		
	Post-IRS	IRS	166	0.1084	71	0.0023
		No IRS	119	0.3782		

3.6 *ANOPHELES* OUTDOOR RESTING RATES

Mean number of outdoor resting mosquitoes was very low and it was not possible perform any further analysis with them. (Table 6)

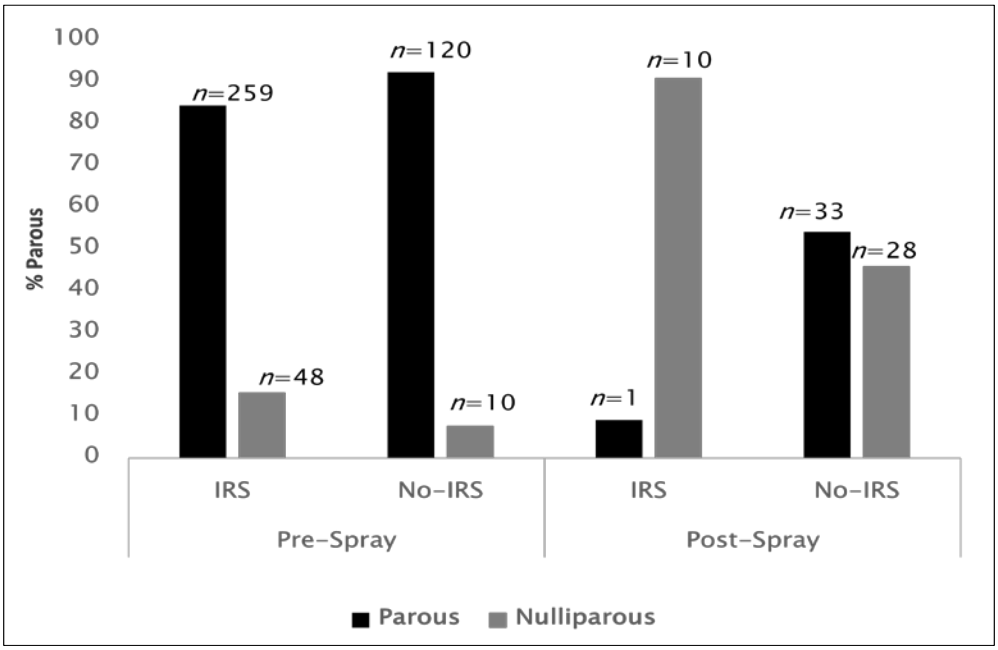
TABLE 6: MEAN NUMBER OF OUTDOOR RESTING ANOPHELES MOSQUITOES COLLECTED FROM OUTDOOR DRUMS

Period	Intention status	Observations	An. funestus mean	An. gambiae mean	Total Anopheles mean	Total Anopheles (95% CI)
Pre-IRS	IRS	118	0.025	0.067	0.093	0.004-0.182
	No-IRS	115	0	0	0	-
Post-IRS	IRS	351	0	0.083	0.083	0.031-0.134
	No-IRS	357	0	0.022	0.022	0.005-0.040

3.7 *ANOPHELES* PARITY STATUS

The *Anopheles* population in both IRS and non-IRS sites were mostly parous in the period before IRS. However, after IRS, the vectors sampled in the IRS sites were mostly young, with over 90% nulliparous, while in the non-IRS sites over 60% were parous (Figure 13). Due to the success of IRS the sample size post IRS in sprayed sites was small.

FIGURE 13: PARITY STATUS OF *ANOPHELES* MOSQUITOES BEFORE AND AFTER IRS IN SPRAY AND NON-SPRAY SITES.



3.8 HUMAN BITING TIMES

A total of 2,046 *Anopheles* mosquitoes were collecetd by HLC over a period of 5 collection nights at 6 different sites conducted both pre-spray (November 2016) and post-spray (June 2017). Samples collected were morpholigically identified as 90.6% *An. funestus*, 6.9% *An. gambaie* s.l., 2.1% *An. coustani* and 0.3% *An. rufipes*. All samples identified as *An. gambiae* s.l. were confirmed by PCR to be *An. arabiensis*. All samples morphologically identified to belong to *An. funestus* group and tested by PCR (n=768) were confirmed to be *An. funestus* s.s. In IRS areas, 98% of all *An. funestus* were collected in the pre-spray period and only 2% post-spray, while in the non-IRS areas, 43% and 57% of *An. funestus* were collected pre- and post-IRS respectively. *An. arabiensis* biting rates were highest in the post-spray period in both IRS and non-spray areas (Table 7).

TABLE 7: NUMBERS OF *ANOPHELES* MOSQUITOES COLLECTED BY HLC PRE- AND POST-IRS, IN THE SPRAY AND NON-SPRAY AREAS (IRS SITES = 100 HOUSES PRE AND POST IRS, NON-IRS = 50 HOUSES*).

*IRS sites = 5 nights, 5 houses per site, 4 sites. Non-IRS SITES = 5 nights, 5 houses per site, 2 sites

		An. funestus			An. gambiae s.l.			An. coustani			An. rufipes		
Spray status	collection location	Pre IRS	Post IRS	Total	Pre IRS	Post IRS	Total	Pre IRS	Post IRS	Total	Pre IRS	Post IRS	Total
IRS	Indoor	391	7	398	20	18	38	0	3	3	0	1	1
	(%)	(98)	(2)	(100)	(53)	(47)	(100)	(0)	(100)	(100)	(0)	(100)	(100)
	Outdoor	211	5	216	13	18	31	19	6	25	0	0	0
	(%)	(98)	(2)	(100)	(42)	(58)	(100)	(76)	(24)	(100)	(0)	(0)	(0)
	Sub total	602	12	614	33	36	69	19	9	28	0	1	1
	(%)	(98)	(2)	(100)	(48)	(52)	(100)	(68)	(32)	(100)	(0)	(100)	(100)
No IRS	Indoor	398	494	892	17	29	46	0	3	3	0	2	2
	(%)	(45)	(55)	(100)	(37)	(63)	(100)	(0)	(100)	(3)	(0)	(100)	(0)
	Outdoor	132	216	348	6	21	27	4	8	12	1	3	4
	(%)	(38)	(62)	(100)	(22)	(78)	(100)	(33)	(67)	(100)	(25)	(75)	(0)
	Sub total	530	710	1240	23	50	73	4	11	15	1	5	6
	(%)	(43)	(57)	(100)	(32)	(68)	(100)	(27)	(73)	(100)	(17)	(83)	(0)

Indoor biting times by *An. funestus* before IRS were similar for sites designated for IRS and control sites, albeit with higher mean biting rates in control sites (Figure 31). *An. funestus* biting began in the early evening at low rates and increased through the night with the highest biting rates occurring between 11:00 pm and 7:00 am. Biting continued throughout the morning albeit at relatively low rates until 11:00 am when collections stopped. In IRS sites, indoor *An. funestus* biting rates after IRS were greatly reduced to near zero (Figure 14). Biting rates by *An. funestus* outdoor were generally far lower than indoors, however, biting times were similar to indoors (Figure 15).

Biting rates of *An. arabiensis* indoors and outdoors were generally far lower than *An. funestus*. Due to the low biting rates, no clear trends could be observed in biting times. However, as with *An. funestus* there were some *An. arabiensis* collected biting indoors and outdoors in the morning between 5:00am and 9:00am (Annex B; Annex, Table 9).

FIGURE 14: BITING TIMES OF *AN. FUNESTUS* S.S. INDOOR, PRE- AND POST-IRS IN IRS AND NON-IRS SITES

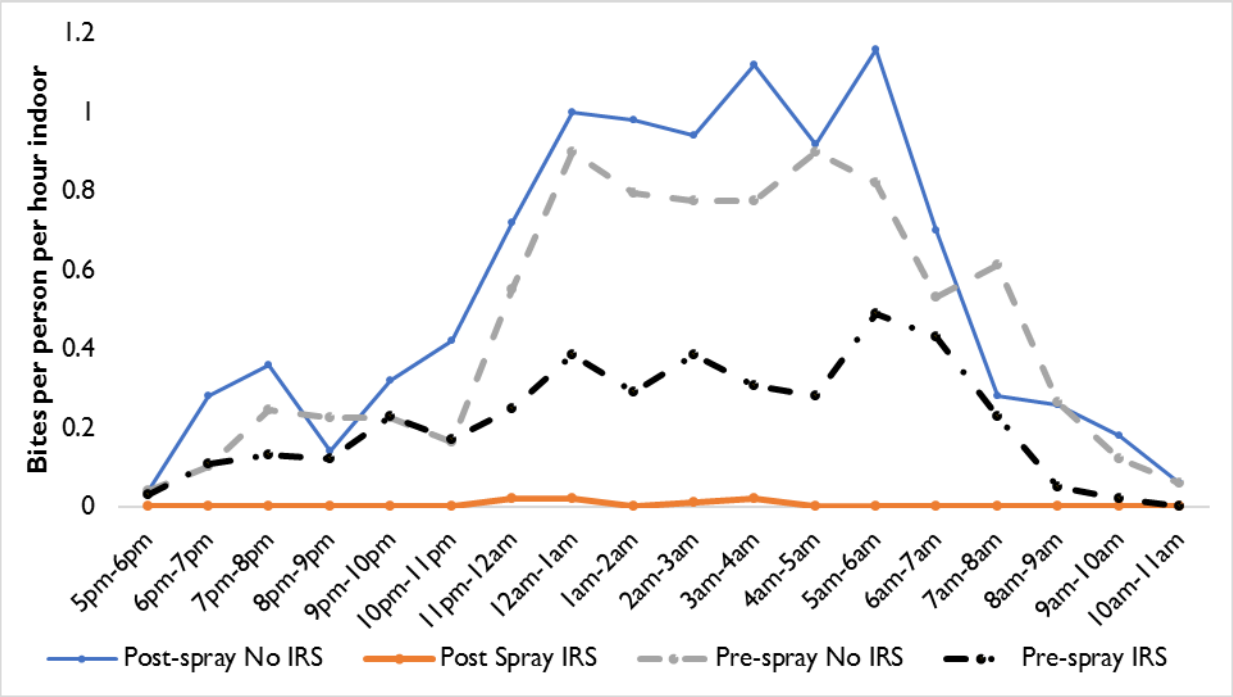


FIGURE 15: BITING RATES OF *AN. FUNESTUS* S.S. OUTDOOR, PRE- AND POST-IRS, IN IRS AND NON-IRS SITES.

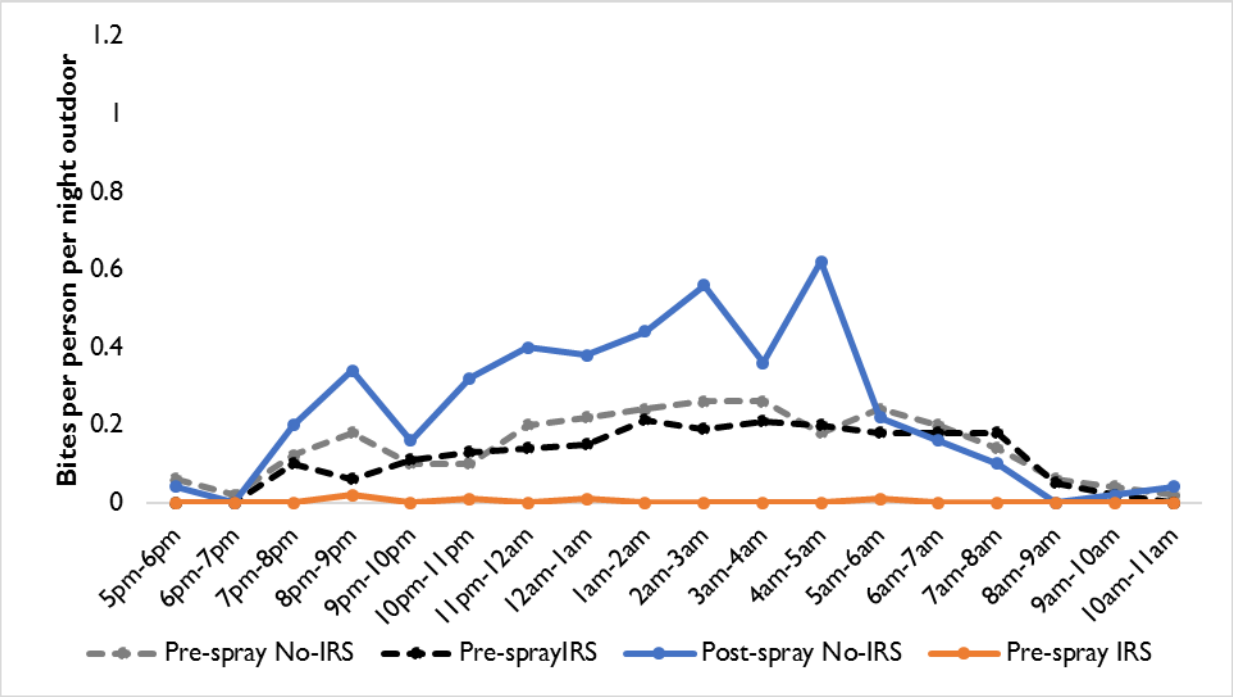
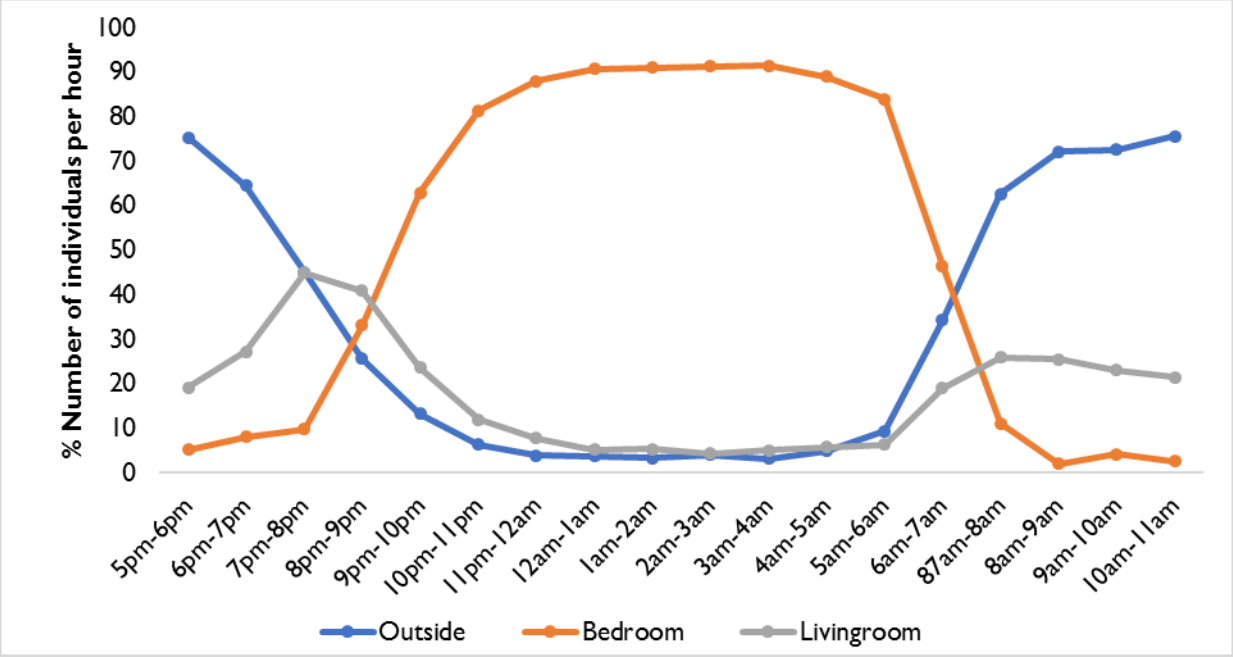


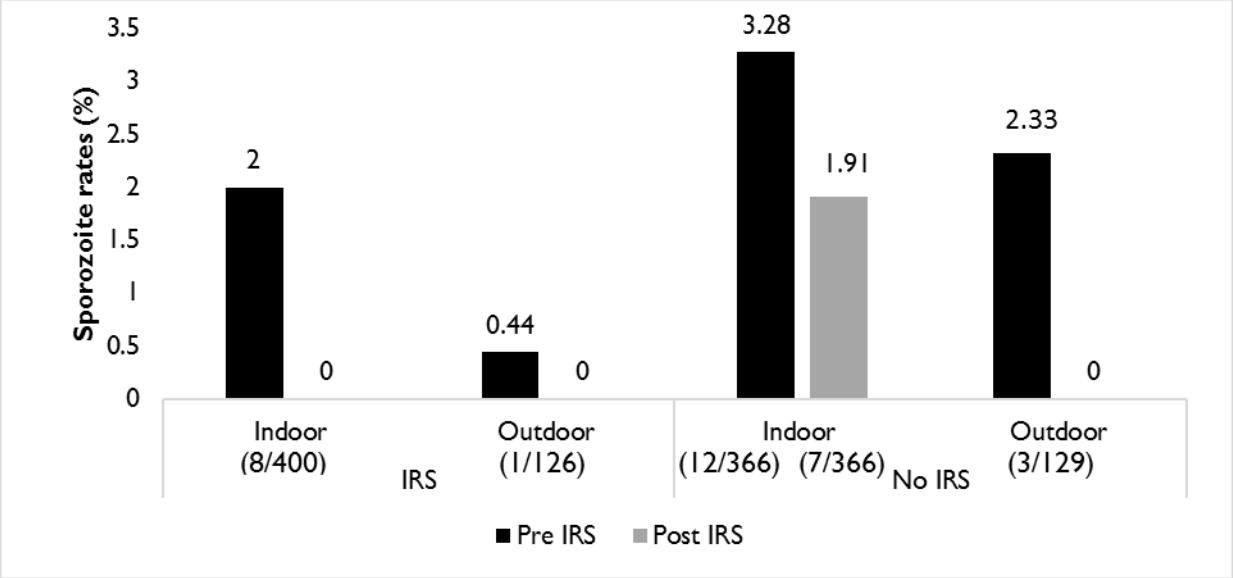
FIGURE 16: DISTRIBUTION OF MEMBERS OF HOUSEHOLD OUTDOORS, AND IN THE LIVING ROOM AND BEDROOM AREAS DURING HUMAN LANDING CATCH.



The HLC collectors observed location of household members over the collection period. For most households, residents spent increasing time indoors from 7:00 pm while the proportion in the living room (unprotected) remained high until 9:00 pm. By 10pm, 90% of householders were located indoors with nearly all residents in the bedroom between 11pm and 5am, meaning that outdoor biting risk during this period was minimal (Figure 16).

A total of 1,695 female *Anopheles* were tested for sporozoite infection with an overall positivity rate of 1.83% (31/1695). Sporozoite infections were detected in *An. funestus* only. The highest sporozoite rates were recorded indoors in the non-IRS regions, with infections detected both pre- and post-IRS. No sporozoite infected *Anopheles* were detected post spray in the IRS region. Sporozoite positive mosquitoes were collected outdoors in the pre-IRS period in both IRS and non-IRS regions (Figure 17).

FIGURE 17: DISTRIBUTION OF SPOROZOITE INFECTION RATES INDOOR AND OUTDOOR, PRE-AND POST- SPRAY BY HUMAN LANDING CATCH COLLECTION.



3.9 RESIDUAL DURATION OF ACTELLIC 300CS®

Over ten months of monitoring insecticide decay rate on sprayed walls, a total of 10,806 susceptible *An. gambiae* s.s. Kisumu stain were exposed to mud and cement walls at different heights in Nyatike, Rongo, Suna West and Uriri Sub Counties. Mortality for control tests on untreated surfaces was negligible at 2%. Wild collected *An. funestus* were not exposed every month due to difficulty of raising sufficient numbers for exposure (Annex, Table 10).

Twenty-four hour mortality remained above 80% for all sites eight months post exposure (Figure 18). Knock-down rates are presented in the Annex D. After nine months mortality in Suna West decreased dramatically to <50%, but was close to 80% in the other three sites (Annex, Figure 32). A comparison of insecticide decay rate between mud and cement walls did not show any clear differences according to substrate (Figure 19). Monthly bioassay monitoring will continue through to the next IRS cycle in February 2018.

FIGURE 18: PERCENT MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* S.S. KISUMU STAIN, 24 HOURS POST EXPOSURE BY MONTH AND SUB COUNTY.

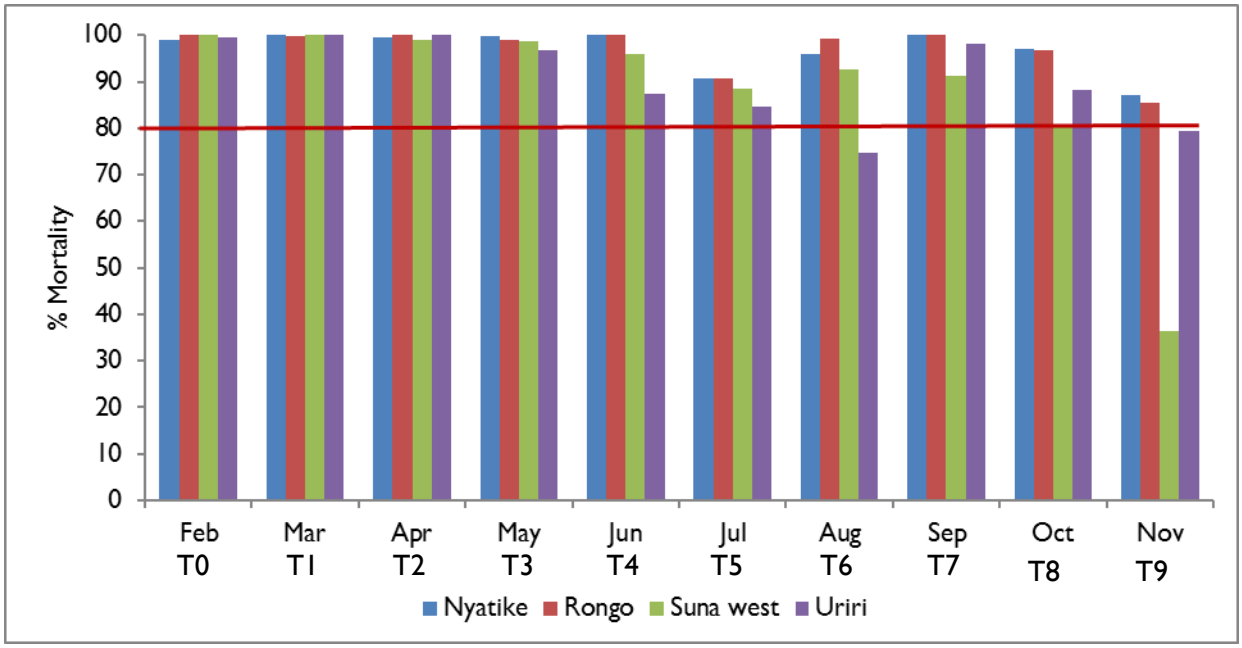
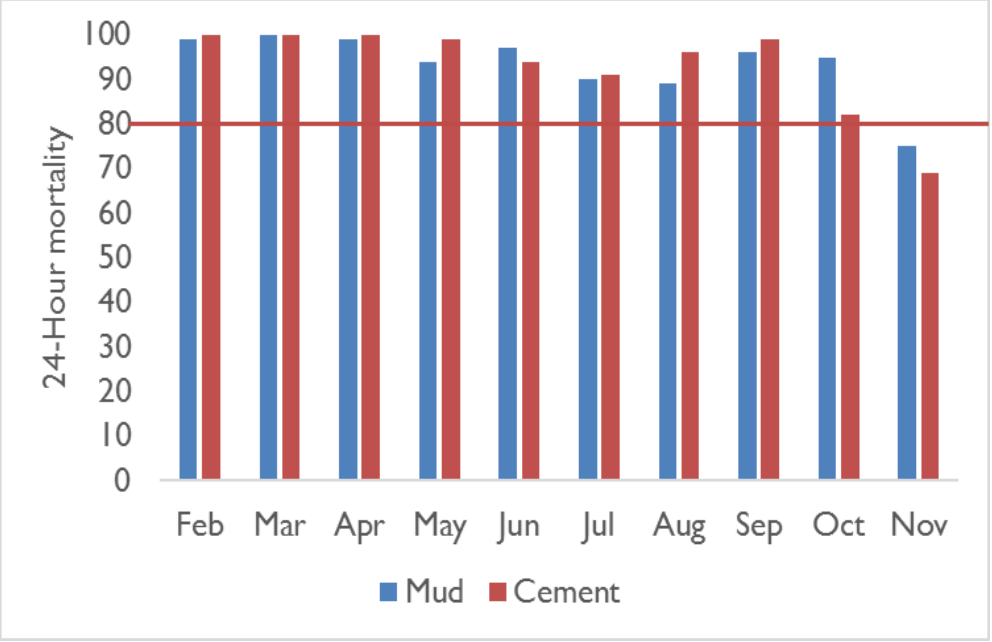


FIGURE 19: COMPARISON OF 24-HOUR MORTALITY OF SUSCEPTIBLE *AN. GAMBIAE* S.S. KISUMU ON MUD AND CEMENT WALLS.



3.10 INSECTICIDE RESISTANCE TESTING

100% mortality of wild F1 *An. funestus* and F1 *An. arabiensis* to pirimiphos-methyl was observed in all sub counties where testing was conducted. Pyrethroid resistance (<90% mortality) was recorded for *An. funestus* in all locations and was generally at a high frequency, with mortality rates particularly low for alphacypermethrin (Figure 20). Pyrethroid resistance (<90% mortality) was also recorded for *An. arabiensis*. However, the frequency of pyrethroid resistance for *An. arabiensis* was generally low for permethrin and deltamethrin, with typical mortality rates >80% (Figure 21).

Dose-response testing of chlorfenapyr in bottle bioassays indicated that 50 mg/ml was the lowest dosage that killed 100% of *An. gambiae* Kisumu, with 25 and 12.5 mg/ml killing <100%. Trends for wild F1 *An. funestus* and *An. arabiensis* were similar, with 50mg/ml killing 100% in Imbo and Rongo; however, 100 mg/ml was needed to kill 100% of *An. arabiensis* in Imbo Sub-county (Figure 22).

Mortality of *An. arabiensis* was slightly less than 100% with the higher 200mg/ml dose of chlorfenapyr in Rongo and Imbo. It has previously been reported that due to the mode of action, mortality rates can become lower at high doses. These results have confirmed 100 mg/ml as a suitable diagnostic concentration for bottle bioassays with chlorfenapyr in Kenya. This dose is the same as that suggested by Dr. Bill Brogdon of CDC, based on previous tests in Zambia.

An. funestus from Imbo (Homabay Sub-County) and Ndhiwa (Ndhiwa Sub-County) sites and the susceptible *An. gambiae* s.s. Kisumu strain, showed full susceptibility to clothianidin five days post exposure (Figure 23). However, mortality in the controls was >20% and tests will be repeated during the next rainy season.

FIGURE 20: PERCENT MORTALITY OF F1 *AN. FUNESTUS* TO PIRIMIPHOS-METHYL, DELTAMETHRIN, PERMETHRIN AND ALPHACYPERMETHRIN IN WHO SUSCEPTIBILITY TESTS (N≈100 PER SITE).

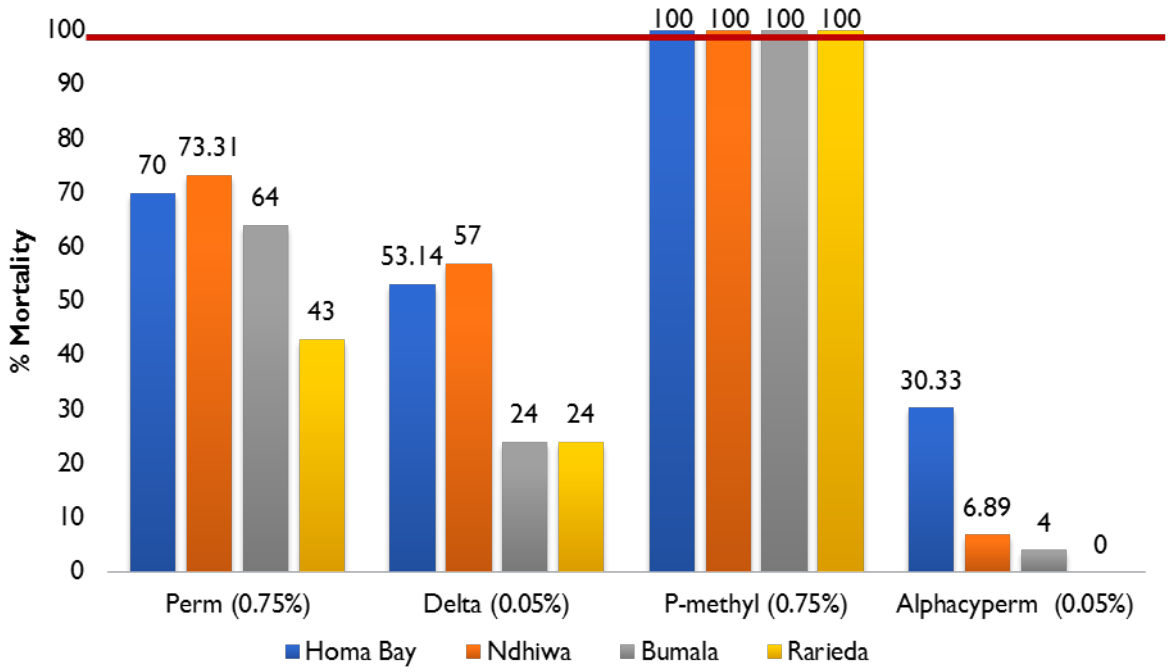


FIGURE 21: PERCENT MORTALITY OF *AN. ARABIENSIS* TO PIRIMIPHOS-METHYL, DELTAMETHRIN, PERMETHRIN AND ALPHACYPERMETHRIN IN WHO SUSCEPTIBILITY TESTS (N≈100 PER SITE).

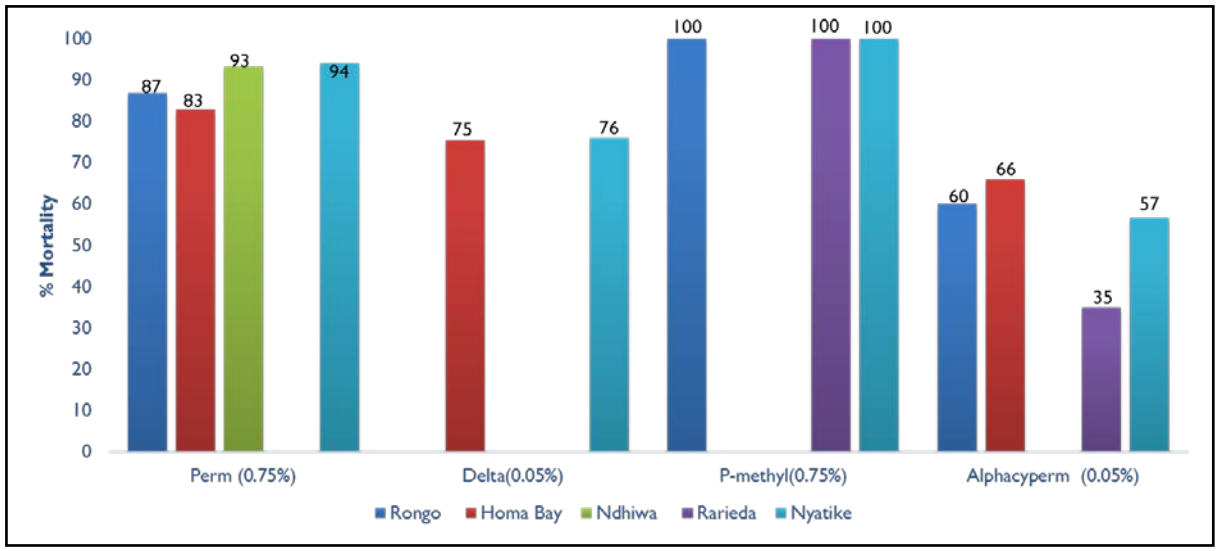


FIGURE 22: PERCENT MORTALITY OF F1 *AN. ARABIENSIS*, *AN. FUNESTUS* AND *AN. GAMBIAE* KISUMU TO VARYING DOSES OF CHLORFENAPYR IN BOTTLE BIOASSAYS (N≈100 PER DOSE PER SITE).

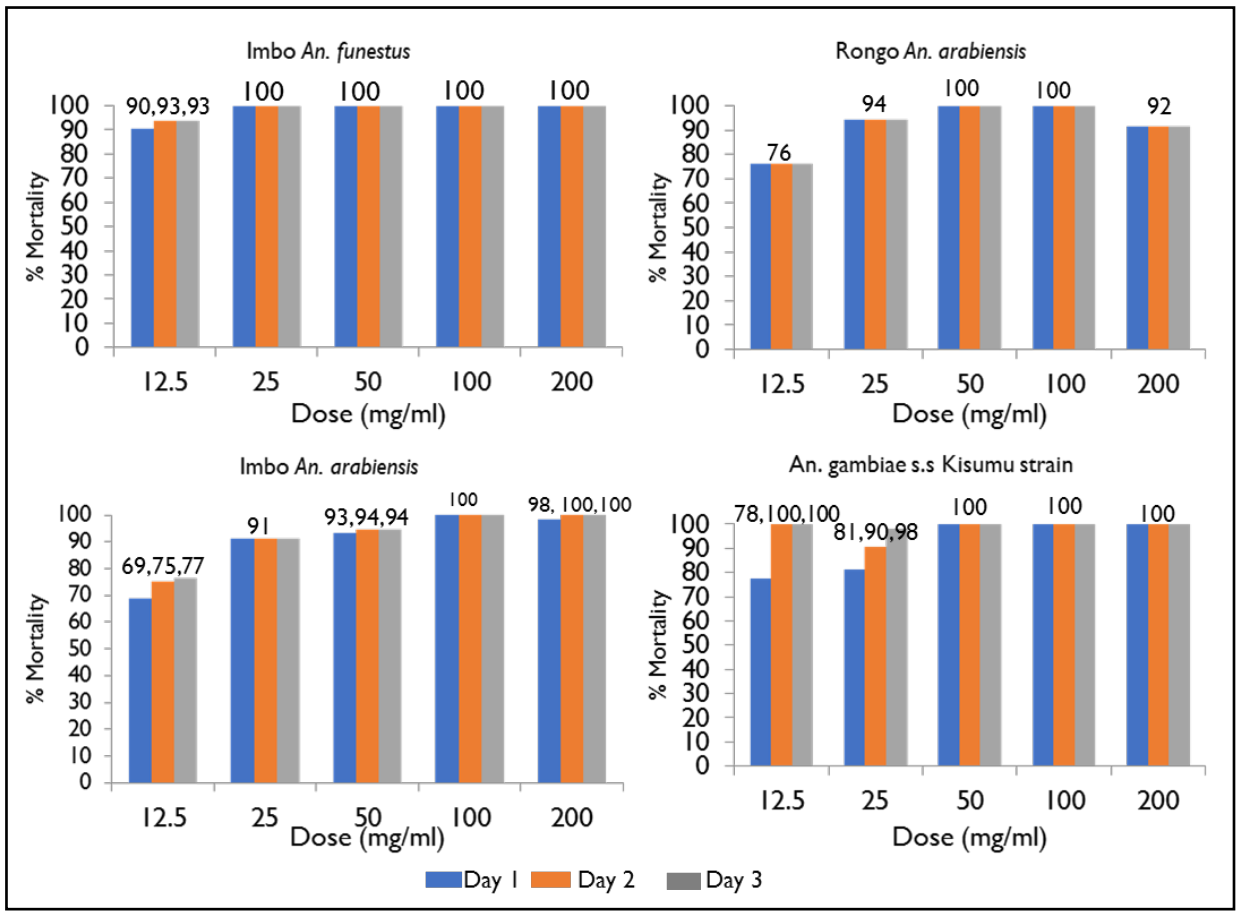
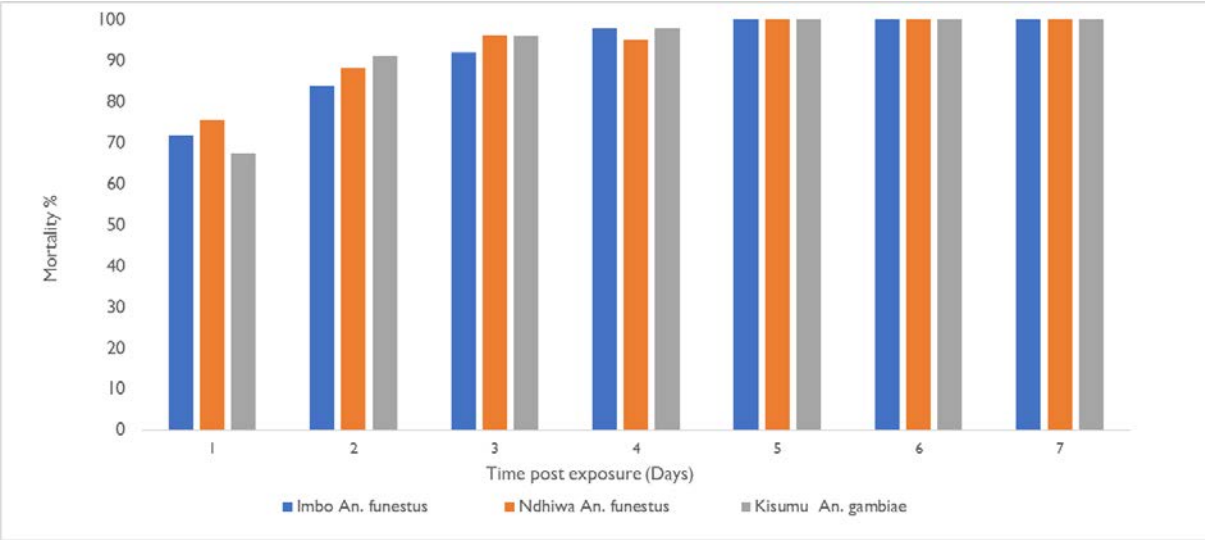


FIGURE 23: PERCENT MORTALITY OF *AN. FUNESTUS* TO 2% W/V OF CLOTHIANIDIN IN WHO SUSCEPTIBILITY TESTS IN COMPARISON TO *AN. GAMBIAE* S.S. KISUMU STAIN (N≈100 PER SITE).



3.11 SPOROZOITE RATE & EIR

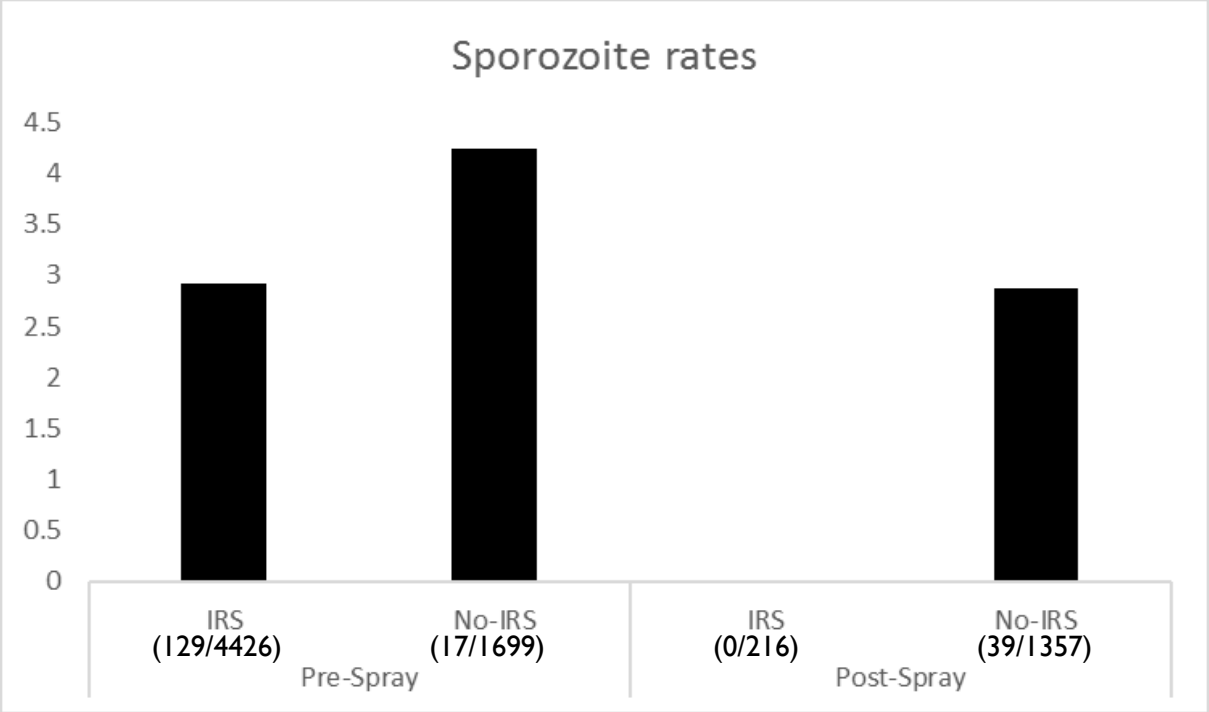
A total of 7,845 *Anopheles* mosquitoes were analyzed for sporozoite infection. Of these, 1,392 were *An. gambiae* s.l. with a sporozoite rate of 0.003 (4/1396) and 6,449 *An. funestus* with sporozoite infection rate of 0.04 (236/6449). From light trap collections, sporozoite rates and EIR varied monthly in the non-IRS sites. In the post-IRS period, *Anopheles* biting rates were lower in the IRS sites as compared to the IRS sites (Table 8). In the post spray period, no sporozoite infection was detected in mosquitoes sampled in IRS sites as compared to non-IRS sites which had mean sporozoite infection rate of over 0.03 (Figure 24). Peaks of high EIRs were associated with periods of known high malaria transmission following wet seasons (Figure 25).

TABLE 8: MONTHLY *ANOPHELES* BITING RATE, SPOROZOITE RATES AND ENTOMOLOGICAL INOCULATION RATE (EIR), IN IRS AND NON-IRS SITES BY CDC LIGHT TRAP

*Nb. Biting rate calculations did not include gravid mosquitoes, assuming they were not host-seeking.

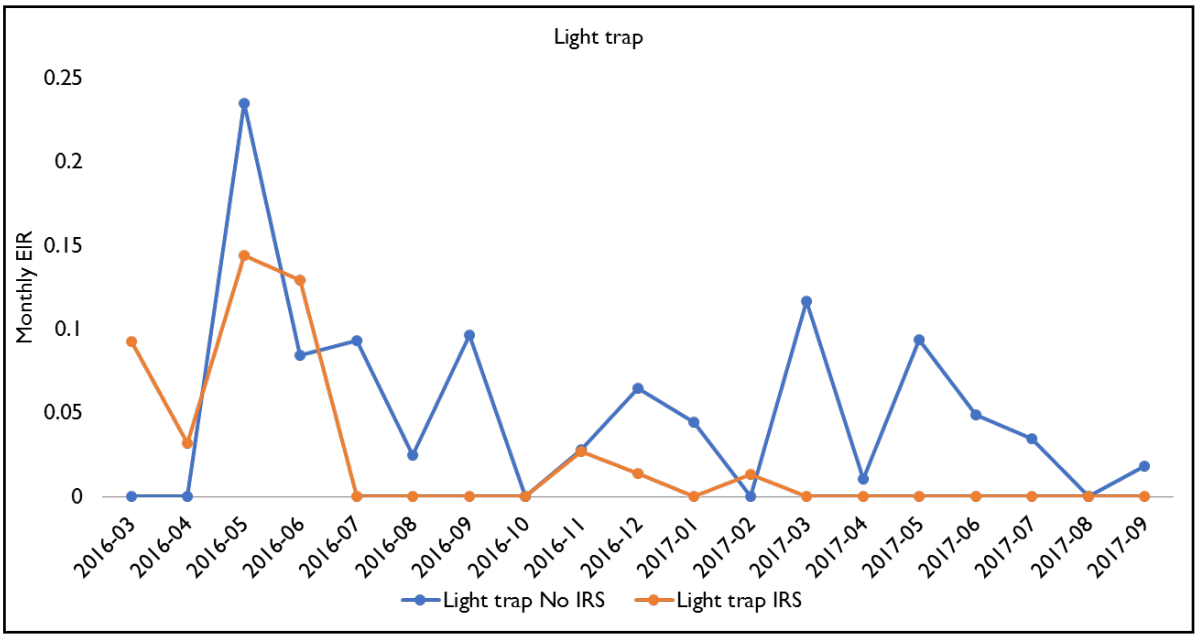
	Light trap, Non-IRS										Light trap, IRS									
		Abdominal status										Abdominal status								
Period	Trap nights	Fed	Gravid	Half Gravid	Unfed	*Biting Rate	Total ELISA Tested	ELISA Pos	Sporozoite rates	EIR	Trap nights	Fed	Gravid	Half Gravid	Unfed	*Biting Rate	Total ELISA Tested	ELISA Pos	Sporozoite rates	EIR
2016-03	18	1	0	0	2	0.167	3	0	0.000	0.000	54	7	1	0	41	0.889	48	5	0.104	0.093
2016-04	20	0	0	0	4	0.200	4	0	0.000	0.000	61	23	3	0	33	0.918	58	2	0.034	0.032
2016-05	38	18	6	1	106	3.289	126	9	0.071	0.235	76	42	16	3	349	5.184	396	11	0.028	0.144
2016-06	37	4	1	0	45	1.324	47	3	0.064	0.085	74	45	6	1	202	3.351	233	9	0.039	0.129
2016-07	67	7	1	0	43	0.746	48	6	0.125	0.093	69	4	4	1	54	0.855	52	0	0.000	0.000
2016-08	78	4	5	3	67	0.949	77	2	0.026	0.025	73	0	2	1	10	0.151	12	0	0.000	0.000
2016-09	77	2	4	0	52	0.701	58	8	0.138	0.097	77	1	1	0	1	0.026	3	0	0.000	0.000
2016-10	54	7	0	0	36	0.796	16	0	0.000	0.000	50	2	0	0	25	0.540	18	0	0.000	0.000
2016-11	52	0	5	5	64	1.327	67	1	0.015	0.020	51	2	0	0	24	0.510	19	1	0.053	0.027
2016-12	61	3	19	2	76	1.328	105	5	0.048	0.063	59	2	16	3	44	0.831	60	1	0.017	0.014
2017-01	55	2	11	1	33	0.655	44	3	0.068	0.045	56	1	5	3	22	0.464	24	0	0.000	0.000
2017-02	56	9	3	1	16	0.464	29	0	0.000	0.000	59	3	9	0	31	0.576	43	1	0.023	0.013
2017-03	60	3	31	1	83	1.450	112	9	0.080	0.117	59	2	0	0	18	0.339	20	0	0.000	0.000
2017-04	59	0	36	4	45	0.831	78	1	0.013	0.011	60	0	3	1	20	0.350	25	0	0.000	0.000
2017-05	59	7	26	1	64	1.220	91	7	0.077	0.094	58	0	11	0	23	0.397	28	0	0.000	0.000
2017-06	58	22	24	0	111	2.293	141	3	0.021	0.049	62	0	9	0	15	0.242	18	0	0.000	0.000
2017-07	54	16	10	1	69	1.593	92	2	0.022	0.035	60	2	3	0	4	0.100	8	0	0.000	0.000
2017-08	58	3	2	0	8	0.190	14	0	0.000	0.000	56	0	0	0	2	0.036	2	0	0.000	0.000
2017-09	55	3	1	0	18	0.382	21	1	0.048	0.018	56	0	1	0	8	0.143	9	0	0.000	0.000

FIGURE 24: SPOROZOITE INFECTION RATES (%) IN IRS AND NON-IRS SITES BEFORE AND AFTER SPRAY



*Parenthesis contain number positive/total number tested

FIGURE 25: MONTHLY ENTOMOLOGICAL INOCULATION RATE (EIR) BY CDC LIGHT TRAP



4. DISCUSSION

The results show a great reduction of indoor host seeking and resting *An. funestus* densities following one round of IRS with pirimiphos-methyl. *An. funestus* was the most dominant vector species in all the sites before IRS with the highest numbers occurring in the intervention sites before spraying. However, after spraying of houses, numbers of the vector reduced to almost undetectable levels. The change in *An. funestus* population densities in the spray sites was detected by all collection methods, PSC, CDC light trap and window exit trap. Furthermore, collections by HLC both indoors and outdoors were so negligible that it was impossible to detect a biting pattern throughout the collection period post spray. These results portray not only a reduction in the indoor occurrence of *An. funestus* but a general reduction in the vector populations in the IRS sites as depicted by the different collection methods. Similar results have been previously observed elsewhere whenever effective vector control methods have been implemented, *An. funestus* has been observed to be most sensitive to effective insecticides. The vector was reduced to near elimination in the Asembo bay area with introduction of pyrethroid treated nets in the early 1990s [12]. Elsewhere in South Africa and Pare/Taveta area of Tanzania and Kenya, there were reports of complete elimination of *An. funestus* following effective IRS campaigns [13]. However, with withdrawal of control, there was a reinvasion of the species in these regions. Recent reports have observed a re-emergence of *An. funestus* in regions where successful interventions, particularly LLINs, had reduced this species to almost undetectable levels [14]. Sustainability of these early gains following a first round of IRS with pirimiphos-methyl is therefore a priority for interruption of malaria transmission in the region. Therefore, it is necessary to monitor the susceptibility of these populations to insecticides and to monitor the duration of efficacy of IRS to ensure high, year-round coverage with this intervention.

The response to IRS of *An. gambiae* s.l., which was predominantly *An. arabiensis*, was quite different from *An. funestus*. Even though *An. gambiae* s.l. occurred in lower numbers as compared to *An. funestus* in the pre-IRS period, only an insignificant reduction in the vector density was realized post IRS and *An. arabiensis* became the most dominant species in the spray areas following application of IRS. This result signifies that *An. arabiensis* was either insensitive to the insecticide used in IRS or the vector species did not have a similar level of exposure to the treated surfaces indoor as did *An. funestus*. Susceptibility testing using standard WHO treated papers showed *An. arabiensis* to be fully susceptible to pirimiphos-methyl, the insecticide used in IRS. This strongly suggests that the low impact of IRS on *An. arabiensis* was not due to insecticide resistance to pirimiphos-methyl, but rather may be the result of some other mechanism such as behavioral resistance. *An. arabiensis* has been associated more with zoophily and exophily in western Kenya [15-17]. The species is also known to be opportunistic in its feeding and considerable numbers are usually collected indoors. The vector therefore accesses the indoor environment to either feed or rest. Our results show a similar trend of *An. arabiensis* in both light trap and PSC collections, which suggest comparable proportions in indoor feeding and resting.

The issue of response of *An. arabiensis* to indoor interventions raises fundamental questions about the species' feeding behavior and actual outdoor densities. Results from a deterministic model reported *An. arabiensis* either to feed outdoors on both humans and cattle, or enter but then rapidly exit houses without fatal exposure to insecticidal nets or IRS [18]. Early exophily is a behavioral phenomenon exhibited by mosquitoes to avoid the lethal effects of the interventions indoors. This behavior possibly explains the low impact of IRS observed on the *An. arabiensis* population in this study. Nonetheless, it is important to note that the highest exiting rate was observed in *An. funestus,* yet the species was most affected by spraying. It is therefore necessary to reconsider other factors leading to proliferation of *An. arabiensis* under an enhanced vector control scenario. Data from a recent investigation in parts of western Kenya reveal that densities of *An. arabiensis* as always reported either from CDC light trap or HLC are only but 'a tip of the iceberg'. Outdoor collection with host decoy trap with cow odor caught over 15 times more *An. arabiensis* as compared to

indoor CDC light trap and 9 times more than outdoor HLC (Abong'o 2017, unpublished data). This observation suggests the existence of a large number of *An. arabiensis* outdoors, feeding on cattle with only a small proportion gaining access into human habitation. This coupled with exiting behavior could offer an explanation into the low impact of IRS against *An. arabiensis* observed in this study. Further investigations are necessary to fully characterize the outdoor vector population in western Kenya.

Data on human biting rates by *Anopheles* mosquitoes provides the most direct measure of individual risk of exposure to malaria infection. The risk of exposure to potentially infectious mosquito bites was highest in the control sites before and after IRS and only before IRS in the intervention sites. A similar biting pattern was observed outdoors although at a lower rate as compared to indoor biting. Spraying houses with pirimiphos-methyl reduced the risk of mosquito bites both indoors and outdoors to almost zero. Most biting by *An. funestus* occurred indoors between 11:00 pm and 6:00 am, a period when most people should be under the protection of their bed nets. However, most early biting and extended biting occurred up to 11:00 am, when most people are away from the protection of their bed nets.

When the only vector control method is LLINs, the presence of *An. funestus* biting early in the evening, and the extension of biting into late morning, presents a worrying situation. Most of such bites would occur while people are away from the protection of bed nets. Few reports of such altered biting behavior of An. *funestus* exist. One such observation has been made in Senegal where broad day light biting *An. funestus* was reported [19]. *Anopheles* mosquitoes are mainly nocturnal in nature and day time biting by *An. funestus* is evidence of behavioral modification that may be attributable to the presence of LLINs. This is a first report of day time biting of *An. funestus* in western Kenya. This phenomenon may contribute to the residual malaria transmission that persists despite widespread LLIN access in the region. The risk of exposure to infectious mosquito bites is seemingly contained by application of IRS. Application of IRS with an effective insecticide demonstrated a great impact in reducing both *An. funestus* numbers and sporozoite rates, thereby lowering the risk of exposure to infectious mosquito bites. Monitoring of vector biting behavior is, however, critical in assessing the risk of malaria transmission with corresponding changes in vector behavior as interventions are enhanced.

Results from cone bioassays show high potency of the insecticide against both the susceptible laboratory colony of *An. gambiae s.s.* Kisumu stain and the wild collected adult *An. funestus* for up to eight months after application, demonstrating the efficacy of this microencapsulated formulation of pirimiphos-methyl. The insecticide formulation showed prolonged activity with the potential to keep mosquito numbers low through the high transmission seasons characterized by long and short rains occurring in western Kenya. Prolonged residual activity of pirimiphos-methyl (Actellic 300CS®) has been previously reported [20] with the promise of providing prolonged control of pyrethroid resistant mosquito and delaying the development of pyrethroid resistance [21]. In addition to Actellic 300CS®, the local vector populations showed susceptibility to two new insecticides, clothianidin and chlofenapyr which are candidates for IRS. The effectiveness of these new classes of insecticide against the local vector population provide options for new tools for vector control to enable rotational application of insecticides for IRS according to the national resistance management strategy [5]. However, it is important that new IRS formulations should be suitably long-lasting to provide protection over the two highest transmission periods in western Kenya.

4.1 CONCLUSION

Application of IRS with Actellic 300CS® for malaria control showed rapid reduction of *An. funestus* numbers in the spray area, reducing the risk of exposure to mosquito bites and malaria transmission. Sustaining these early gains in malaria control in western Kenya is important to transmission reduction the region. The insignificant impact of IRS on *An. arabiensis* is likely due to the vector's feeding behavior. We hypothesize that a large number of *An. arabiensis* exist in the outdoor environment, feeding mostly on cattle with occasional blood meals from humans both indoors and outdoors, hence sustaining malaria transmission. Further investigations are needed to fully

characterize the outdoor *Anopheles* population in the region and additional control measures other than LLINs and IRS might be recommended.

ANNEX A: *ANOPHELES* SEASONALITY PER SUB COUNTY (INDOOR CDC-LT & PSC)

An. funestus was the predominant species in CDC light trap collections in Awendo, Nyatike, Suna West, Uriri and Rongo Sub Counties before IRS in March 2017. Pre-IRS biting peaks were generally observed between December and February (short rainy season) and April to July (long rainy season). However, a substantial decrease in the mean number of *An. funestus* per trap was observed in the period after IRS (Figure 26). While *An. funestus* densities dropped to almost undetectable levels in sprayed areas, CDC light traps continued to collect *An. gambiae* s.l. albeit at very low densities (Figure 26). A similar trend was observed for indoor resting densities with a remarkable decrease in the number of *An. funestus,* while that of *An. gambiae* s.l. remained above zero post spray (Figure 27).

In the unsprayed sub counties of Homa Bay, Ndhiwa and Kuria, *An. funestus* was the dominant vector in both light traps (Figure 28) and PSCs (Figure 29) throughout the collection period. High biting rates were recorded in unsprayed districts between March and July 2017 during the long rainy season. Further analysis of *Anopheles* densities is presented in section 3.2.2.

FIGURE 26: MONTHLY MEAN NUMBER INDOOR HOST SEEKING *AN. FUNESTUS* AND *AN. GAMBIAE* S.L. IN IRS SUB COUNTIES PRE- AND POST-IRS (COLLECETD BY CDC LIGHT TRAP).

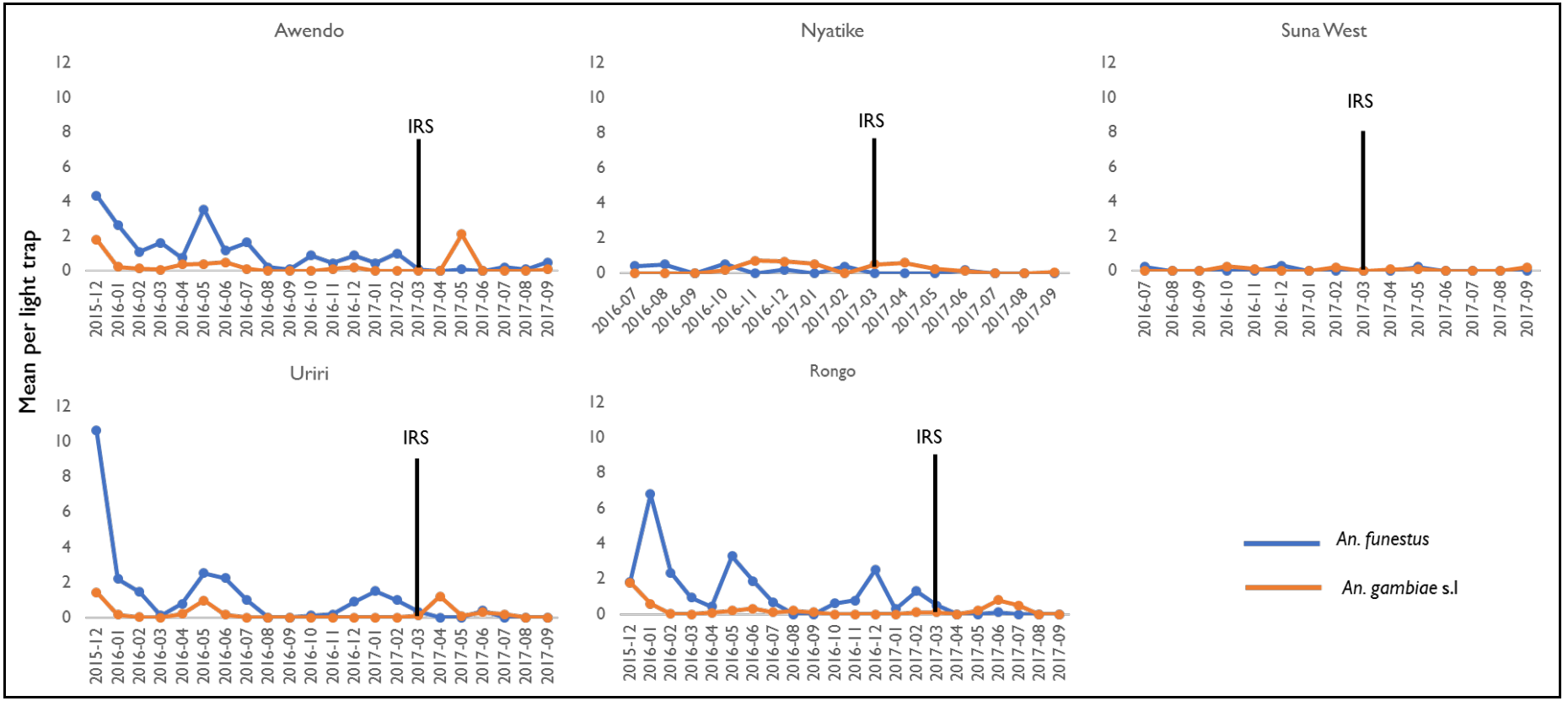


FIGURE 27: MONTHLY MEAN NUMBER OF INDOOR RESTING *AN. FUNESTUS* AND *AN. GAMBIAE* S.L. IN IRS SUB COUNTIES PRE- AND POST-IRS (COLLECTED BY PSC).

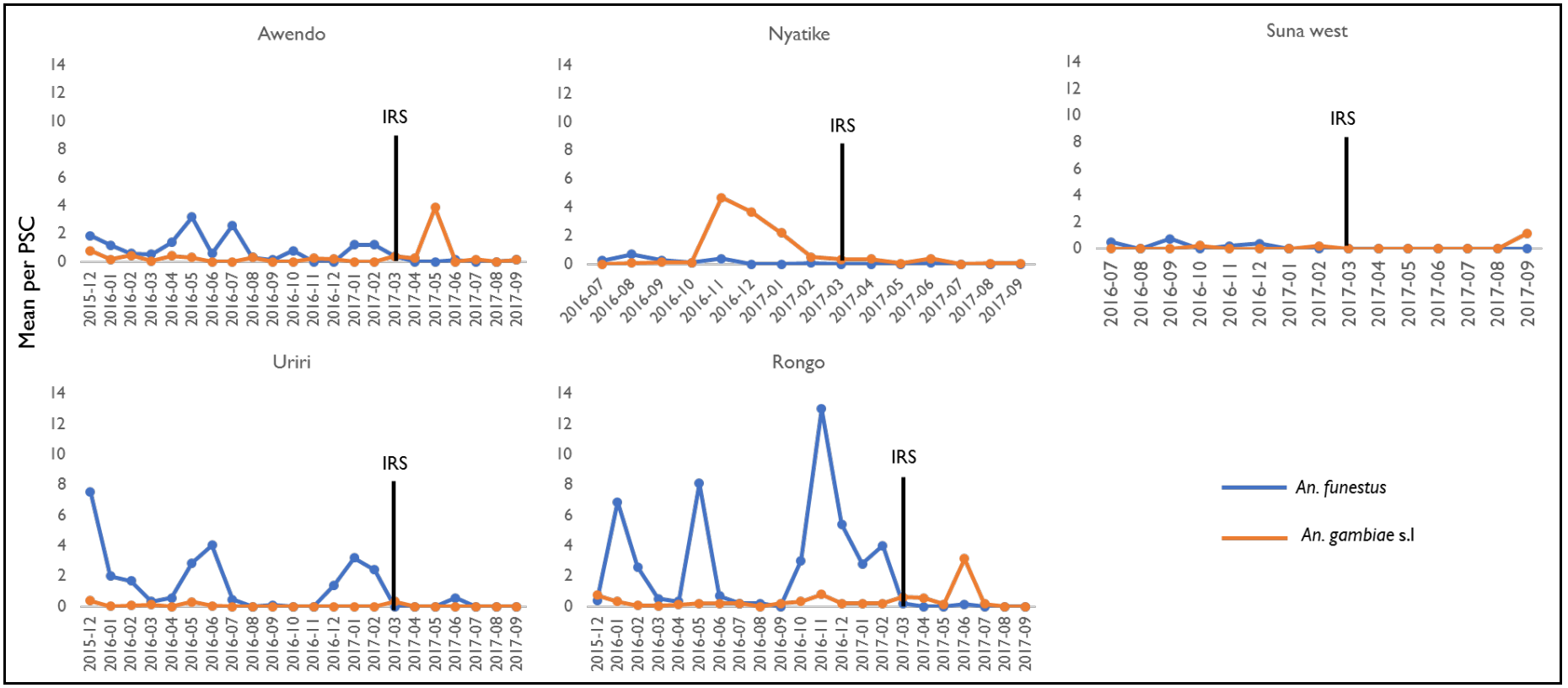


FIGURE 28: MONTHLY MEAN NUMBER OF INDOOR HOST-SEEKING *AN. FUNESTUS* AND *AN. GAMBIAE* S.L. IN NON-IRS SUB COUNTIES PRE- AND POST IRS (COLLECTED BY CDC LIGHT TRAP).

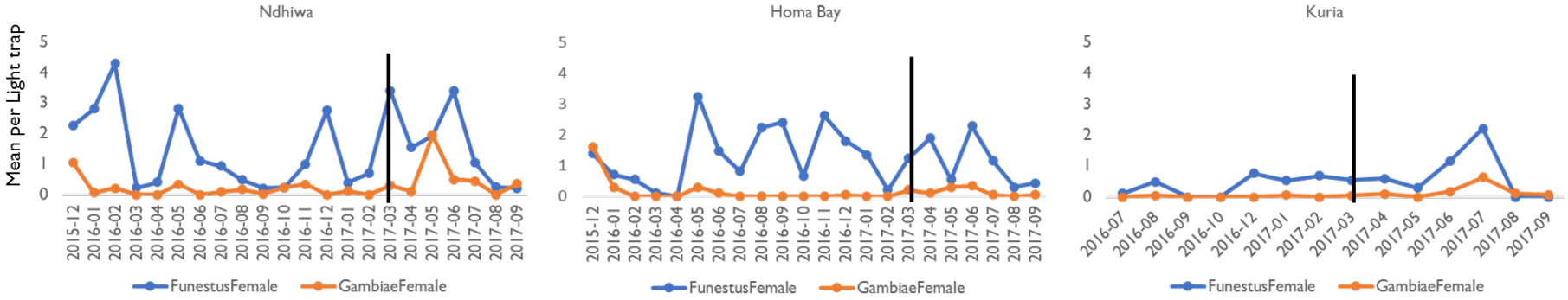
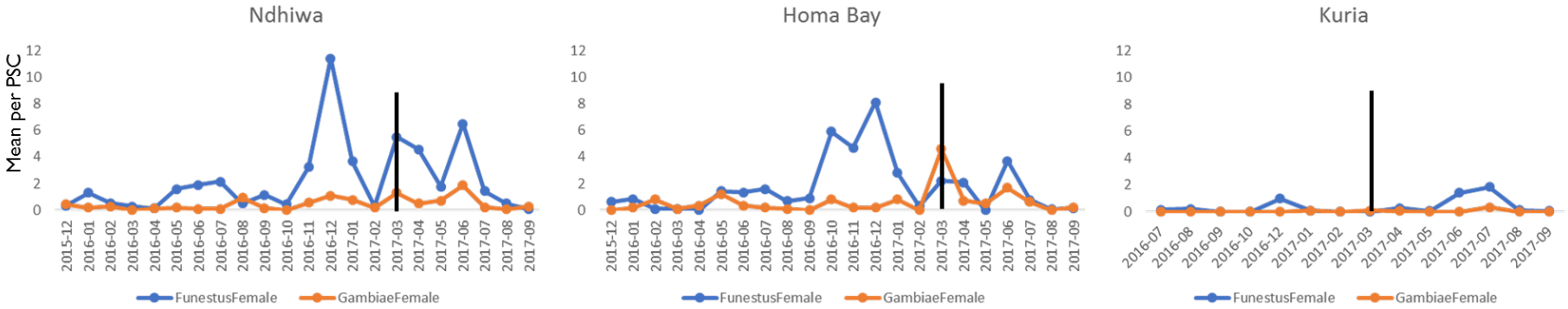


FIGURE 29: MONTHLY MEAN NUMBER OF INDOOR RESTING *AN. FUNESTUS* AND *AN. GAMBIAE* S.L. IN NON-IRS SUB COUNTIES PRE- AND POST-IRS (COLLECTED BY PSC).



ANNEX B: BITING RATES OF *AN. ARABIENSIS*

FIGURE 30: BITING RATES OF *AN. ARABIENSIS* INDOOR, PRE- AND POST-IRS, IN IRS AND NON-IRS SITES.

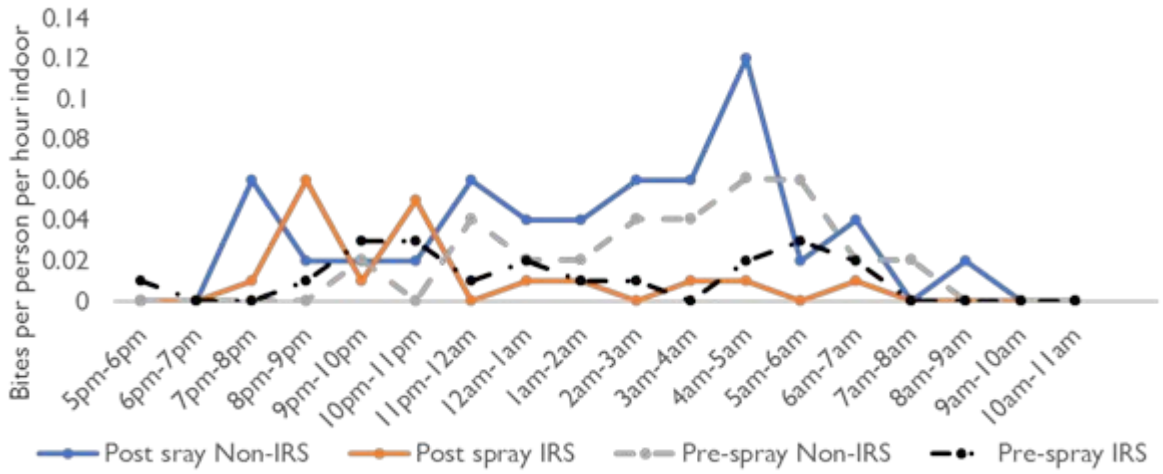
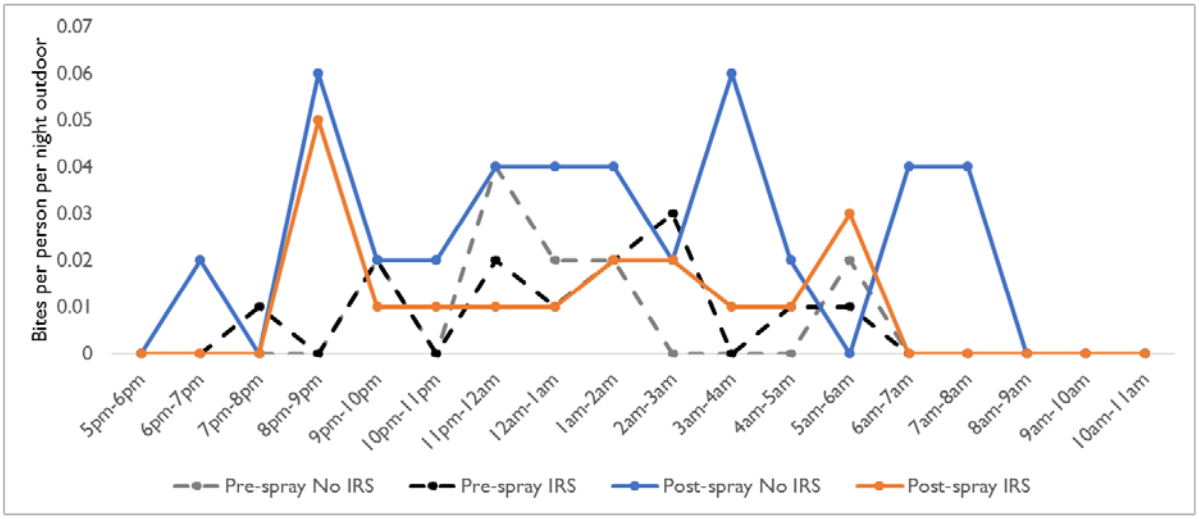


FIGURE 31: BITING RATES OF *AN. ARABIENSIS* OUTDOOR, PRE- AND POST-IRS, IN IRS AND NON-IRS SITES.



ANNEX C: ANOPHELES BITING RATES BY HUMAN LANDING CATCH (HLC)

TABLE 9: BITING RATES OF *AN. FUNESTUS* AND *AN. GAMBIAE* COLLECTED BY HUMAN LANDING CATCH (HLC) INDOOR AND OUTDOOR PRE- AND POST-IRS

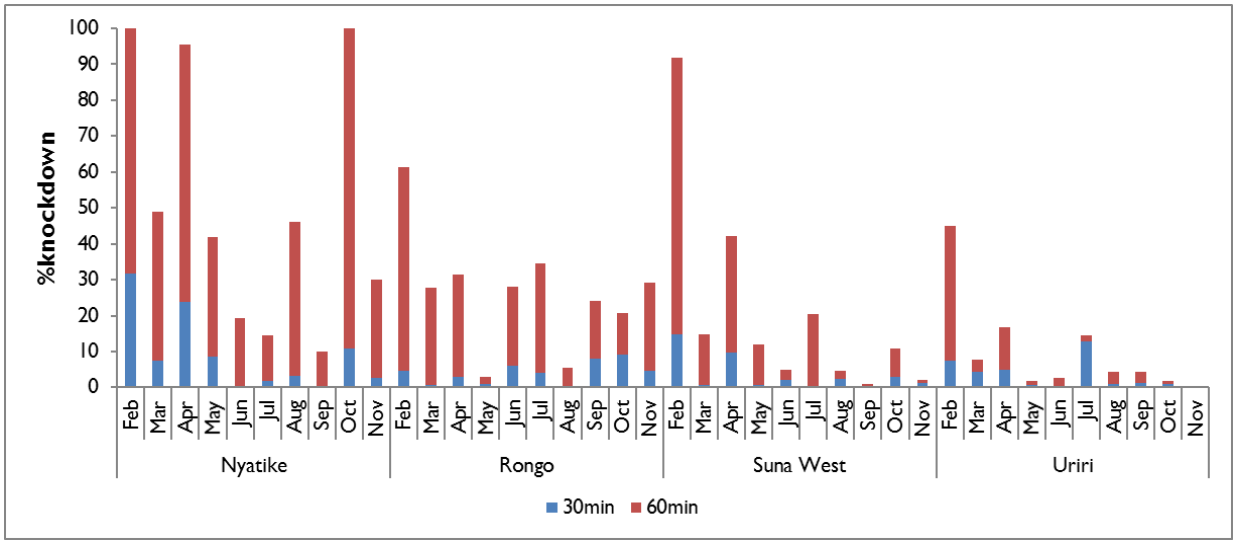
				An. funestus		*An. arabiensis*	
Intervention	**Period**	**Location**	**Person night**	**Number collected**	**Biting rates**	**Number collected**	**Biting rates**
IRS	Pre-IRS	Indoor	100	391	3.91	20	0.2
		Outdoor	100	211	2.11	13	0.13
	Post-IRS	Indoor	100	7	0.07	18	0.18
		Outdoor	100	5	0.05	18	0.18
No IRS	Pre-IRS	Indoor	50	398	7.96	17	0.34
		Outdoor	50	132	2.64	6	0.12
	Post-IRS	Indoor	50	494	9.88	29	0.58
		Outdoor	50	216	4.32	21	0.42

ANNEX D: RESIDUAL EFFICACY OF ACTELLIC 300CS®

TABLE 10: NUMBERS OF SUSCEPTIBLE *AN. GAMBIAE S.S.* KISUMU STRAIN AND WILD COLLECTED *AN. FUNESTUS* EXPOSED TO DIFFERENT WALL TYPES IN FOUR SUB COUNTIES.

		An. gambiae s.s. Kisumu strain							Wild An. funestus	
		Test					Control			
		Height			24 Hrs mortality					
Sub county	Wall type	0.5	1	1.5	No.	% Mortality	No	24 Hrs mortality	No.	24 Hrs mortality
Nyatike	Mud	646	624	628	1812	95	599	12	310	303
	Cement	219	209	219	638	99	220	4	122	122
Rongo	Mud	694	691	691	2043	98	692	12	16	16
	Cement	300	300	290	864	97	298	4	10	10
Suna west	Mud	560	560	560	1464	87	558	19	190	168
	Cement	300	300	300	786	87	280	8	40	40
Uriri	Mud	625	624	627	1747	93	628	12	0	0
	Cement	280	280	279	745	89	280	4	0	0
Total		3624	3588	3594	10099	93.46	3555	75	688	659

FIGURE 32: PERCENT KNOCK DOWN AT 30 MIN AND 60 MIN POST-EXPOSURE OF SUSCEPTIBLE *AN. GAMBIAE* S.S. KISUMU STRAIN BY MONTH OF EXPOSURE AND SUB COUNTY.



ANNEX E: REFERENCES

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